**INSTITUTO POLITÉCNICO DE LISBOA**

Instituto Superior de Engenharia de Lisboa
Escola Superior de Tecnologia da Saúde de Lisboa

# Myocardial Perfusion Scintigraphy: Impact of Anxiety on Image Quality

Catarina Marques de Carvalho

Final Master's Work to obtain the degree of
Master's in Biomedical Engineering

**Supervised by:**
Lina da Conceição Capela de Oliveira Vieira, *PhD* - (ESTeSL/IPL)

**September 2023**

**INSTITUTO POLITÉCNICO DE LISBOA**

Instituto Superior de Engenharia de Lisboa
Escola Superior de Tecnologia da Saúde de Lisboa

# Myocardial Perfusion Scintigraphy: Impact of Anxiety on Image Quality

Catarina Marques de Carvalho

Final Master's Work to obtain the degree of
Master's in Biomedical Engineering

**Supervised by:**
Lina da Conceição Capela de Oliveira Vieira, *PhD* - (ESTeSL/IPL)

**Jury:**
**President**: Paulo Jorge Leitão Pessoa Guerreiro, PhD - (ESTeSL/IPL)
**Vogal**: Carlos Gerardo Einisman, PhD – (Hospital de Oncologia "Maria Curie", Argentina)
Edgar Lemos Pereira, IPL Specialist – (*NuclearMed*)
Lina da Conceição Capela de Oliveira Vieira, PhD - (ESTeSL/IPL)

**September 2023**

# Resumo

As doenças cardiovasculares (DCV) são um grupo de doenças do coração e dos vasos sanguíneos, e a principal causa de mortalidade em todo o mundo, afetando milhões de pessoas todos os anos, estimando-se 17,9 milhões de vidas a cada ano (1–3). A Doença Arterial Coronária (DAC) é a forma mais comuns de DCV, sendo responsável por mais de 40% das mortes na Europa (4,5). Na DAC, as artérias do paciente ficam estreitadas por placas de ateroma ou formação de trombos, devido à formação de placas de colesterol (6,7). A obstrução da placa leva ao comprometimento do fluxo sanguíneo, diminuindo o fornecimento de oxigênio ao miocárdio (2,6).

A Cintigrafia de perfusão do miocárdio (CPM) é um método de imagem bem estabelecido para diagnóstico e avaliação de DAC (8). Esta técnica foi desenvolvida em 1970 e desde então tem sido muito utilizada em cardiologia e outras áreas de tratamento médico (3). Em Portugal, representa 47% da totalidade dos exames de medicina nuclear realizados para diagnósticos (9).

A CPM é um método de imagem médica que usa tomografia computadorizada por emissão de fotão único (SPECT, sigla inglesa de *Single Photon Emission Computed Tomography*), sincronizado com um sinal de eletrocardiograma (Gated) (3,4,10,11). No seu procedimento, é realizada a administração intravenosa de radiofármacos em duas fases distintas (esforço e repouso), o que leva a tempos de aquisição relativamente longos, exigindo a cooperação do paciente para obter um estudo de imagem com qualidade diagnóstica (12).

A CPM está associada a uma elevada carga emocional por se tratar de um exame com características específicas, nomeadamente: a) duração e posicionamento durante o exame; b) falta de conhecimento; c) uso de radiação ionizante e d) resultados dos exames. Visto que este procedimento pode diagnosticar doenças potencialmente fatais, os pacientes apresentam ansiedade antes, durante e após o exame (13,14). Altos níveis de ansiedade podem comprometer a qualidade da imagem obtida por desencadear movimentos involuntários e voluntários do paciente durante a aquisição da imagem. Estes movimentos podem aumentar o aparecimento de artefactos na imagem, com consequentes implicações na sua interpretação. A movimentação do paciente pode afetar cerca de 10-26% dos estudos de CPM (15,16).

Várias estratégias não farmacológicas foram introduzidas antes e durante procedimentos de imagem para minimizar a ansiedade do paciente, como meditação, massagem, aromaterapia, hipnose e uso de música (17–20). A intervenção musical é uma estratégia não farmacológica indolor, confiável, de baixo custo e sem efeitos colaterais (21,22). Pode ajudar a maximizar os esforços para promover o conforto e relaxamento do paciente, uma vez que a atenção dos pacientes em aspetos como tempo do procedimento ou sintomas, pode ser desviada à medida que se concentram na música (23).

O principal objetivo deste projeto foi avaliar o impacto da ansiedade do paciente na qualidade das imagens da cintigrafia de perfusão do miocárdio. Para alcançar o objetivo principal, foram cumpridos vários objetivos intermédios através da análise de parâmetros psicológicos, bioquímicos, fisiológicos e sociodemográficos da amostra.

O presente projeto incluiu dois estudos, um estudo Cross-Sectional e um estudo Piloto, em pacientes que realizaram CPM, protocolo 1 dia repouso-esforço, por indicação clínica. O estudo Cross-Sectional, incluiu uma amostra de 63 participantes, e foi usado para caracterizar o impacto da ansiedade do paciente na qualidade das imagens de CPM. O estudo Piloto, com uma amostra de 34 participantes, foi usado para avaliar o impacto da intervenção musical e a sua eficácia na diminuição da ansiedade dos pacientes quando realizam CPM. O estudo Cross-sectional foi realizado na NuclearMed, no Hospital Particular de Almada. O estudo Piloto foi um estudo multicentro realizado no serviço de Medicina Nuclear (MN) da Clínica Joaquim Chaves de Miraflores (n=23) e no serviço de MN do Hospital de Santa Maria (n=11).

Para a análise das amostras dos dois estudos foram utilizados como ferramentas de medição parâmetros sociodemográficos, psicológicos, bioquímicos e fisiológicos. Estas medições foram aplicadas em 5 momentos distintos do exame: T0, 2 dias antes do exame; T1, na chegada ao serviço; T2, antes da aquisição de imagens de repouso; T3, depois da aquisição de imagens de esforço; T4, antes da aquisição de imagens de esforço; T5, depois da aquisição de imagens de esforço. Para o estudo Piloto apenas foram realizados os tempos do T2 ao T5. Como parâmetros sociodemográficos do paciente, utilizamos a idade, género, habilitações literárias, número de exames de imagem médica realizados anteriormente, medicação e grupos terapêuticos, número de fatores de risco e os seus tipos. Como parâmetros psicológicos utilizamos dois formulários de medição de ansiedade, STAI-S, sigla inglesa de *State-Trait Anxiety Inventory for Adults* e VAS, sigla inglesa de *Visual Analogue scale*. A escala STAI-S, ou STAI forma Y-1, medida nos tempos T1, T2 (no caso do estudo Piloto) e T5, é uma forma específica da STAI em que utiliza apenas os parâmetros de avaliação “state”, estado do participante no momento da avaliação. É composto por 20 itens e classificado entre 0 e 4 onde 0 é “Nada” e 4 é “Muito”. A escala VAS é uma escala de dor ou ansiedade com apenas um item e classificado entre 0 “Nada ansioso” e 10 “Extremamente ansioso”. Como parâmetros bioquímicos, medidos do T0 ao T3 no estudo Cross-sectional e no T2 e T3 para o estudo Piloto, utilizamos o biomarcador de ansiedade, Cortisol. Foram feitas recolhas de saliva dos pacientes e posteriormente doseadas. Como parâmetros fisiológicos, medidos do T1 ao T5 no estudo Cross-sectional e do T2 ao T5 no estudo Piloto, foram utilizados tensão arterial, frequência cardíaca, frequência respiratória e saturação de oxigénio ($SpO_2$%).

Para a análise dos resultados estes foram divididos em três secções: análise do estudo Cross-sectional, análise do estudo Piloto e comparação dos dois estudos.

Os resultados do estudo Cross-sectional mostraram que parâmetros sociodemográficos como a idade, género e habilitações literárias podem ter influência na ansiedade do paciente.

Na análise de qualidade de imagem foram encontradas diferenças estatisticamente significativas entre o ruído médio da imagem com o parâmetro fisiológico: batimentos cardíacos. Verificamos assim que quanto maior os valores de batimentos dos pacientes maior o ruído médio apresentado nas suas imagens. Para além disso verificamos que de acordo com o parâmetro psicológico STAI-S conforme o aumento dos scores dos pacientes, ocorreu um aumento do número de movimentos <1 pixel dos pacientes mostrando assim que, a ansiedade teve influência em movimentos dos pacientes inferiores a 1 pixel.

No estudo Piloto verificamos que os temas musicais específicos: “La fille aux cheveux de lin”, Debussy and “Pachelbel D major” foram os mais escolhidos pelos participantes, com uma média de volume de 60dB. Ao comparar os resultados do estudo Cross-sectional com o estudo Piloto verificamos que de acordo com os parâmetros psicológicos ocorreu uma diminuição de scores na amostra do estudo Piloto em todos os momentos do exame, mostrando assim que existiu uma influência da intervenção musical na diminuição da ansiedade dos pacientes.

Tanto no estudo Cross-sectional como no estudo Piloto, apesar de existir uma diminuição de valores de concentração dos parâmetros bioquímicos ao longo do exame, as concentrações foram muito baixas comparativamente aos valores de referência na literatura (24). Para além disso, as concentrações variam ao longo do dia e desta forma, podem afetar e alterar os nossos resultados, influenciando posteriormente a sua análise e interpretação.

Considera-se o presente estudo como mais um passo no desenvolvimento de métodos de melhoramento para exames de imagem médica em específico à cintigrafia de perfusão do miocárdio e podemos concluir que parâmetros fisiológicos como a frequência cardíaca tem influência na qualidade de imagem e o número de movimentos dos pacientes é influenciado pela sua ansiedade durante exames CPM. É importante continuar a estabelecer novos parâmetros de medição para estes testes, e fazer estudos com amostras de maior dimensão para se obter resultados estatisticamente mais significativos. Para além disso, continuar a aprofundar os benefícios da utilização de intervenções musicais neste tipo de exames de imagem médica.

**Palavras-chave**: Cintigrafia de perfusão do miocárdio, Qualidade de imagem, Ansiedade, Intervenção Musical

# Abstract

Myocardial perfusion scintigraphy (MPS) is a well-established imaging method for diagnosing and evaluating ischemic heart disease (8). This imaging technique represents 47% of the total number of nuclear medicine exams performed in Portugal (9). MPS uses intravenous administration of radiopharmaceuticals in two distinct phases (stress and rest), which leads to relatively long acquisition times, requiring patient cooperation to obtain a diagnostic-quality imaging study (12). MPS is associated with a high emotional burden since it is an exam with specific characteristics, namely: a) duration and positioning during the exam; b) lack of knowledge; c) use of ionizing radiation and d) exam results. Since this procedure can diagnose a life-threatening disease, patients experience anxiety before and during the exam (13,14). High levels of anxiety may compromise the quality of the image obtained by triggering involuntary and voluntary movements of the patient during the image acquisition. These movements may enhance the appearance of artifacts in the image, with consequent implications in its interpretation. Patient movement may affect 10-26% of MPS studies (15,16). Several non-pharmacological strategies have been introduced before and during imaging procedures to minimize patient anxiety. Musical intervention is a painless, reliable, low-cost and without side effects non-pharmacological strategy (21,22). It can help maximize efforts to promote the patient's comfort and relaxation; since patients' attention on aspects such as procedure time or symptoms can be diverted as they focus on the music (23).

The main objective of this project was to assess to evaluate the impact of patient's anxiety on the quality of myocardial perfusion scintigraphy images. The project was carried out in CPM exams with a 1-day rest-stress protocol and divided into two types of study: Cross-Sectional Study with a total of n=63 participants and a Pilot Study, with a total of n=34 participants. In the Cross-sectional study, the sample and image quality were analysed in accordance with the main objective of the project. In the Pilot study, the sample was analysed, and a musical intervention was used to understand its effectiveness in reducing patients' anxiety. Sociodemographic, psychological, biochemical, and physiological parameters were used as measurement tools, applied at 6 moments of the exam: T0, T1, T2, T3, T4 and T5.

We observed in the Cross-sectional study that the % noise is influenced by the patients' heart rate and that the number of patients movements <1 pixel increased with the increase in STAI-S scores. In the Pilot study we found that the specific musical themes: "La fille aux cheveux de lin", Debussy and "Pachelbel D major" were the most chosen by participants, with an average volume of 60dB. When comparing the results of the Cross-sectional study with the Pilot study, we found that, according to psychological parameters, there was a decrease in scores in the Pilot study sample at all times of the exam. We can conclude that physiological parameters such as patients' heart rate and the number of movements are influenced by their anxiety during MPS exams.

**Keywords**: Myocardial perfusion scintigraphy, Image Quality, Anxiety, Musical Intervention

# Acknowledgments

To all the people involved in this project. To Professors Ana Grilo, Ana Pires, Marina Almeida, Elisabete Carolina, Luísa Veiga, and Catarina Ginete for all the support and learning they gave me. To Professor Jaime Reis for his support, dedication, and flexibility in helping me.

From the services where I carried out this project, I would like to thank from HPA Edgar Pereira, Ana Correia and Ana Malaia, Maria and Matilde, the other students in internship, Doctor Luís Oliveira, and ladies from the administration for all their support and monitoring. They were excellent professionals and contributed greatly to the development of this study. From the Santa Maria Hospital service to technologist Raquel and the other technologists and professionals for their support and flexibility. From Clínica Joaquim Chaves to Srª Ana for her patience and kindness, again to Doctor Luís for all his support in this phase of the project and to the service technologists.

I would like to thank my parents, Fernanda and Pedro, for all the support and patience they gave me. You are the best, it wouldn't be possible without you, I'm proud to be your daughter, I'm always learning from you. To my sister Mafalda for all her affection and concern, for always believing in me and having me as an example. To my grandparents, Eugénia, Amélia and Francisco for all the support they gave me, their affection, patience, and concern.

To my boyfriend Rui for always being there. Believing in myself from the beginning and in moments of frustration not letting me give up, always being a safe place. To his family also for all their affection and predisposition.

To my friends, despite the distance, they are always there when I need them. Thank you also to my cats Mimi and Chiquinha and my dog FIFA. They always know when to keep me company and comfort me.

I would also like to thank Mr. João from the ISEL secretariat. He was always very attentive during my master's degree, always paying a lot of attention and care to help with my problems.

Lastly, I would like to give a very special thank you to my supervisor and professor Lina Vieira. Not only as an example of a teacher, but she is also an example of a human being. She was tireless throughout my journey, I always felt supported. Always very kind, affectionate and throughout the project, she contributed a lot to my personal and professional growth, always transforming my failures into learning and never into frustration. With great respect and great pride in having guided by you, a big thank you.

# Contents

**Resumo** ..... i
**Abstract** ..... iv
**Acknowledgments** ..... v
**Contents** ..... vi
**List of Figures** ..... ix
**List of Tables** ..... xii
**List of Appendices** ..... xiii
**List of abbreviations and acronyms** ..... xiv
**1. Introduction** ..... 1
1.1 Framework ..... 1
1.2 Objectives ..... 2
1.3 Dissertation Structure ..... 2
**2. The Myocardium** ..... 3
2.1 Structure and functions ..... 3
2.2 Vital signs ..... 5
2.2.1 Blood Pressure ..... 5
2.2.2 Heart rate ..... 6
2.2.3 Respiratory rate ..... 7
2.2.4 Blood Oxygen Levels ..... 8
**3. Cardiovascular diseases** ..... 9
3.1 Background ..... 9
3.2 Coronary Heart Disease ..... 9
3.3 Cardiovascular risk factors and medication ..... 10
**4. Medical Imaging Procedures** ..... 13
4.1 Background ..... 13
4.2 SPECT ..... 14
4.2.1 Background ..... 14
4.2.2 Gamma Cameras ..... 16
4.2.3 Radiopharmaceuticals ..... 18
4.3 Myocardial Perfusion Scintigraphy ..... 20
4.3.1 Background ..... 20
4.3.2 Patient Preparation ..... 20
4.3.3 Protocol ..... 21

**5. Image Quality** ..... 23
5.1 Image quality characterization ..... 23
5.1.1 Spatial Resolution ..... 23
5.1.2 Noise ..... 24
5.1.3 Contrast ..... 24
5.2 Factors that influence image quality ..... 24
**6. Anxiety** ..... 26
6.1 Background ..... 26
6.2 Neurobiological mechanisms of anxiety ..... 26
6.3 Measurement parameters to anxiety ..... 28
6.3.1 Psychological parameters ..... 28
6.3.2 Anxiety Biomarker: Cortisol ..... 29
6.4 Influence of anxiety in medical imaging ..... 31
**7. Musical Intervention** ..... 33
**8. Methods** ..... 35
8.1 Study Protocol ..... 35
8.2 Sample ..... 36
8.2.1 Sample information ..... 36
8.2.2 Participants Recruitment ..... 36
8.2.3 Inclusion and Exclusion criteria ..... 38
8.3 Measurement parameters ..... 39
8.3.1 Socio-demographic parameters ..... 39
8.3.2 Psychological parameters ..... 39
8.3.3 Biochemical parameters ..... 40
8.3.4 Physiological parameters ..... 45
8.4 Image acquisition characteristics ..... 46
8.5 Image Quality ..... 47
8.5.1 Noise ..... 48
8.5.2 Contrast ..... 49
8.5.3 Number of movements ..... 49
8.6 Musical Intervention ..... 50
8.7 Study dependent and independent variables ..... 52
8.8 Statistical Analysis ..... 52
**9. Results** ..... 53
9.1 Introduction ..... 53
9.2 Cross-sectional study ..... 54

9.2.1 Sociodemographic parameters ..... 54
9.2.2 Psychological parameters: STAI ..... 57
9.2.3 Psychological parameters: VAS ..... 62
9.2.4 Biochemical parameters ..... 66
9.2.5 Physiological parameters ..... 71
9.2.6 Image Quality ..... 74
9.3 Pilot Study ..... 79
9.3.1 Measurement parameters ..... 79
9.3.2 Musical Intervention ..... 80
9.4 Cross-Sectional study and Pilot study comparations ..... 82
**10 Discussion** ..... 85
**11 Conclusions** ..... 93
**12 Limitations of study** ..... 94
**13 References** ..... 95
**14 Communications and Publications** ..... 109
14.1 Oral communications ..... 109
14.2 Publications ..... 110
**APPENDICES** ..... 111

# List of Figures

**Figure 1:** General structure and flow of blood through the heart..... 3
**Figure 2:** The cardiac conduction system and ECG ..... 4
**Figure 3**: Legend: Pulmonary and Systemic Circulatory Systems. Schematic diagram showing serially connected pulmonary and systemic circulatory systems and the direction of blood flow; RA, Right atrium; RV, right ventricle; LA, left atrium, LV, left ventricle. ..... 5
**Figure 4:** Coronary artery disease: normal artery and artery obstructed by cholesterol plaque B) Atherosclerosis stages in coronary artery disease, caused by cholesterol plaque..... 10
**Figure 5:** Key steps in an emission tomography study ..... 13
**Figure 6**: Standard SPECT imaging display. A - The short-axis images represent a portion of the anterior, lateral, inferior, and septal walls. B - Vertical long-axis images represent the anterior wall, apex, and inferior wall. C - Horizontal long-axis images represent the septum, apex, and lateral walls. ..... 15
**Figure 7**: Cardiac plane definition and display for tomographic imaging modalities .... 15
**Figure 8:** Schematic diagram of a conventional gamma camera used in SPECT. ..... 16
**Figure 9:** Schematic of a gamma camera detector ..... 17
**Figure 10:** Schematic diagram of a scintillation detector comprising a scintillation material coupled to a photomultiplier tube. ..... 17
**Figure 11**: One (A) and two (B) day rest-stress Tc99m imaging protocols. ..... 21
**Figure 12:** Anatomical illustration of important areas of the limbic system ..... 26
**Figure 13**: The neural structure of the Papez circuit ..... 27
**Figure 14**: Schematic view of the major anterior neuronal circuits (fronto-temporal connectivity) of the limbic system mostly connected to the frontal and temporal cortices. ..... 27
**Figure 15**: Cortisol structure ..... 29
**Figure 16**: Competitive ELISA procedure. ..... 31
**Figure 17**: Steps of 1-day protocol for MPS exams. ..... 35
**Figure 18:** Salivette structure and Information. The salivette contains, a blue lid, a paper label: white/blue and a roll of synthetic fibers for determining cortisol from saliva. ..... 40
**Figure 19**: Hygienic collection of saliva samples for diagnosis and therapeutic control. ..... 41
**Figure 20**: Cortisol Competitive ELISA Kit. ..... 41

**Figure 21:** Diagram of the dilutions of the standards. ..... 43
**Figure 22**: ROIs to the zone of interest and background. Three myocardial projections of one patient. ..... 48
**Figure 23**: Xeleris 4.0 Processing and Review Workstation Software| GE Healthcare data. A) “Motion Correction” data of one participant; B) Number of movements in one participant. ..... 49
**Figure 24**: Number of participants (%) in Cross-sectional study and Pilot study. ..... 53
**Figure 25:** Distribution of female and male participants in different age groups. ..... 54
**Figure 26:** Comparation between participants gender by carry out previous imaging exams. ..... 55
**Figure 27**: Different levels of previous exams difficulty and the number of participants. ..... 55
**Figure 28:** Number of CVRFs and its types compared with number of participants in each. ..... 56
**Figure 29:** Number of different medication and its types comparing with the number of participants in each. ..... 57
**Figure 30**: STAI-S scores do different exam moments ..... 58
Figure 31: STAI-S scores comparing with participant’s gender. ..... 59
**Figure 32**: STAI-S scores compared with participants prior imaging exams. ..... 59
**Figure 33:** STAI-S scores with participants habilitation. ..... 60
**Figure 34:** STAI-S scores compared with CVRFs and medication treatment groups. ..... 61
**Figure 35**: VAS scores for different exam moments. ..... 62
**Figure 36**: VAS scores with participant’s gender. ..... 63
**Figure 37**: VAS scores compared with participants prior imaging exams. ..... 64
**Figure 38:** VAS scores with participants academic degree ..... 64
**Figure 39:** VAS scores compared with CVRFs and medication treatment groups. ..... 65
**Figure 40**: Cortisol concentrations to different moments of exam. ..... 67
**Figure 41**: Cortisol concentrations in different moments of the exam comparing the number of participants with different schedules. ..... 68
**Figure 42**: Comparison between collection time and cortisol concentrations, for each exam moment. ..... 68
**Figure 43**: Cortisol concentrations in T1 comparing with participant’s gender. ..... 69
**Figure 44**: Cortisol concentrations in T1 comparing with participant’s age. ..... 70
**Figure 45**: Measurements of physiological parameters for different moments of the exam ..... 71
**Figure 46**: Hearts rate values comparing with participant´s age. ..... 72

**Figure 47**: Blood pressure compared with CVRFs and the number of medication treatment group. ..... 73
**Figure 48**: Participant´s previous imaging exams compared with number of movements <1. ..... 74
**Figure 49**: Patients movements <1 and between 1-2 compared with psychological parameters. ..... 75
**Figure 50**: Comparison between Noise (%) and psychological parameters, for 3 heart projections, SA, VLA and HLA. ..... 76
**Figure 51**: %Noise for the 3 different heart projections. ..... 76
**Figure 52**: Correlation between contrast and psychological parameters (STAI-S and VAS) to 3 different heart projections, SA, VLA and HLA. ..... 77
**Figure 53**: Participants distribution in NM services, for Pilot study. ..... 79
**Figure 54:** Participants gender distribution in different age groups. ..... 79
**Figure 55**: Cortisol concentrations in Pilot study sample to the two measurement moments in exam. ..... 80
**Figure 56:** Different musical choices to compared with number of participants. ..... 80
**Figure 57:** Musical choice in different participant´s age groups. ..... 81
**Figure 58**: Musical volume compared with participant's age. ..... 81
**Figure 59**: Comparison between psychological scores and different acquisition times. ..... 82
**Figure 60:** Comparison between physiological parameters from Cross-sectional study and Pilot study. ..... 83
**Figure 61**: Comparison between psychological parameters from Cross-sectional study and Pilot study. ..... 84

# List of Tables

**Table 1**: Stages of Blood Pressure ..... 6
**Table 2**: Average heart rates for different ages and genres ..... 7
**Table 3:** Normal RR values in different ages ..... 7
**Table 4**: Gradation of oxygen saturation levels ..... 8
**Table 5:** Radionuclides used in SPECT imaging. min, minutes; h, hours; d, days; y, years. EC, electron capture; IT, isomeric transition; b+, positron decay; β, beta decay ..... 19
**Table 6:** Male and female salivary cortisol levels in different age-groups. All means (m) are adjusted for differences in age and proportions are standardized for age. Δ-cortisol - logarithmic morning salivary cortisol – logarithmic evening saliva cortisol. Cortisol concentrations in nmol/L. ..... 30
**Table 7**: Contents and storage from ELISA Kit ..... 42
**Table 8**: Blood pressure equipments specifications ..... 45
**Table 9**: Image acquisition characteristics used in the 3 different NM services. ..... 47
**Table 10**: Characteristics of the 3 gamma cameras used in the study. ..... 47
**Table 11**: Headphones equipment specifications ..... 50
**Table 12:** Minimum, maximum, and mean of participants age in the Cross-sectional study and Pilot study. ..... 53
**Table 13:** Different ages groups with number of male and female participants ..... 54
**Table 14**: Correlation between STAI-S values and VAS values, for moment T1 in the Cross-sectional study sample. ..... 66
**Table 15**: Correlation between STAI-S values and VAS values, for moment T5 in the Cross-sectional study sample. ..... 66
**Table 16:** Correlation between biochemical parameters and psychological parameters, in the Cross-sectional study sample. ..... 70
**Table 17:** Statistic results to psychological, physiological, and biochemical parameters in Cross-sectional study. ..... 73
**Table 18:** Statistic results to image quality compared with physiological parameters to Cross-sectional study. ..... 78

# List of Appendices

APPENDIX 1: Informed consent for participants. ..... 111
APPENDIX 2: Sociodemographic questionnaire. ..... 112
APPENDIX 3: STAI-S questionnaire. ..... 113
APPENDIX 4: VAS questionnaire. ..... 114
APPENDIX 5: Sociodemographic data for Cross-sectional study. ..... 115
APPENDIX 6: STAI-S values for Cross-sectional study. ..... 116
APPENDIX 7: STAI-S statistic results to Cross-sectional study. ..... 117
APPENDIX 8: VAS scores for Cross-sectional study. ..... 118
APPENDIX 9: VAS statistic results to Cross-sectional study. ..... 119
APPENDIX 10: Cortisol data for Cross-sectional study. ..... 120
APPENDIX 11: Cortisol concentrations and standards. ..... 121
APPENDIX 12: Cortisol statistic results to Cross-sectional study. ..... 124
APPENDIX 13: Physiological results for Cross-sectional study. ..... 125
APPENDIX 14: Physiological statistic results. ..... 126
APPENDIX 15: Image quality data to Cross-sectional study. ..... 127
APPENDIX 16: Image quality statistic results to Cross-sectional study. ..... 128
APPENDIX 17: Socio-demographic data for Pilot Study. ..... 129
APPENDIX 18: STAI-S scores to Pilot study. ..... 130
APPENDIX 19: VAS scores for Pilot Study. ..... 131
APPENDIX 20: Physiological data for Pilot study. ..... 132
APPENDIX 21: Statistic results to Pilot study. ..... 133

# List of abbreviations and acronyms

**$^{123}$I –** Iodine 123

**$^{125}$I –** Iodine 125

**$^{131}$I –** Iodine 123

**$^{111}$In –** Indium 111

**$^{177}$Lu –** Lutetium 177

**$^{99}$Mo –** Molybdenum 99

**$^{99m}$Tc –** Technetium 99m (metastable)

**3D –** Tridimensional

**ACE** - Angiotensin-converting enzyme

**ARBs** - Angiotensin Receptor Blockers

**ARNIs** - Angiotensin Receptor-Neprilysin Inhibitors

**ASCN -** American Society of Nuclear Cardiology

**BB** – Beta blockers

**BMI -** Body mass index

**BP** – Blood Pressure

**CAD** – Coronary artery diseases

**CCB** - Calcium Channel Blockers

**CHD** – Coronary heart diseases

**CSS** – Cross-sectional study

**CVDs** - Cardiovascular diseases

**CVRFs** - Cardiovascular risk factors

**Dbp** - Diastolic blood pressure

**ECG** – Electrocardiogram

**ELISA** - Enzyme-linked immunosorbent assay

**ESTeSL** - *Escola Superior de Tecnologia da Saúde de Lisboa*

**ET** - Emission tomography

**FHH** - Family Health History

**FOV** – Field-of-view

**GABA** - g-amino-butyric-acid

**GC** – Gamma Camera

**GLM** - General linear model

**HBP** – High blood pressure

**HLA** – Horizontal long axis

**HPA**- *Hospital Particular de Almada*

**HR** – Heart rate

**Kg** – Kilogram

**LDL** - Low-density lipoprotein

**LEHR** - Low-energy high-resolution

**LV** - Left ventricle

**$m^2$** - Square meter

**MBq** – Megabecquerel

**mmHg** - millimetres of mercury

**MPS** – Myocardial Perfusion Scintigraphy

**mSv** - Millisievert

**NaI(Tl)** - Thallium-doped Sodium Iodine Crystal

**NM** – Nuclear Medicine

**PAD -** Peripheral artery diseases

**PET** – Positron emission tomography scan

**PHA -** Pulse-Height Analyzer

**Pixel** - picture element

**PMHR** - Predicted maximum heart rate

**PMTs** – Photomultiplier Tubes

**PS** – Pilot study

**QRS** – QRS complex

**ROI** - Region of Interest

**RR**- Respiratory rate

**SA** – Short Axis

**Sbp** - Systolic blood pressure

**SPECT** - Single photon emission computed tomography scan

**SPL** - Sound Pressure Level

**$SpO_2$** - Oxygen saturation

**STAI-S** - State-Trait Anxiety Inventory form

**T2DM** – Type 2 Diabetes Mellitus

**TIA** - Transient ischemic attacks

**THR -** Target heart rate

**VAS** - Visual analogue scale

**VLH** – Vertical long axis

# 1. Introduction

## 1.1 Framework

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide, affecting millions of people every year (1). Coronary Artery Disease (CAD) is the most common form of CVD, accounting for more than 40% of deaths in Europe (4,5).

MPS is a well-established non-invasive imaging method, using a Single Photon Emission Computed Tomography (SPECT) and is synchronized with the electrocardiogram (Gated) signal (4,10). MPS diagnose and stratify the risk of CAD (4,8,25). In Portugal, MPS is the most frequent conventional Nuclear Medicine (NM) examination, representing almost 47% of all conventional NM procedures (1).

MPS is associated with a high emotional burden since it is an exam with specific characteristics, namely: a) duration and positioning during the exam; b) lack of knowledge; c) use of ionizing radiation and d) exam results. High levels of anxiety may compromise the quality of the image obtained by triggering involuntary and voluntary movements of the patient during the image acquisition. These movements may affect 10-26% of MPS studies (13,15,16) and enhance the appearance of artifacts in the image, with consequent implications in its interpretation.

Assessing patients' anxiety during these types of exams and understanding it has been the work of many recent studies on MPS exams (20,26,27). By developing and identifying the problem, analysing it quantitatively and qualitatively, makes it simpler to subsequently search for new strategies to reduce patients' anxiety and thus the probability of artifacts and consequent implications on image quality.

Several non-pharmacological strategies have been introduced before and during imaging procedures to minimize patient anxiety. (17–20). Musical intervention is a painless, reliable, low-cost and without side effects non-pharmacological strategy (21,22). It can help maximize efforts to promote the patient’s comfort and relaxation; since patients' attention on aspects such as procedure time or symptoms can be diverted as they focus on the music (23).

## 1.2 Objectives

The main objective of this project was to assess the impact of patient anxiety on the quality of myocardial perfusion scintigraphy images.

In order to achieve the purpose of this dissertation, the following objectives were defined:

- To assess the impact of patient anxiety on the quality of myocardial perfusion scintigraphy images;
- To evaluate anxiety in patients undergoing MPS by psychological, physiological and biochemical methods;
- To identify the relationship between anxiety levels and the number of repetitions of MPS exams;
- To evaluate the relation between anxiety levels and MPS image quality (e.g., noise, contrast, and sensitivity);
- To assess the effectiveness of listening to music in the decreasing of anxiety in patients undergoing MPS using musical methods.

## 1.3 Dissertation Structure

This dissertation is structure into 14 main chapters.

The first chapter corresponds to the Introduction, which provides a framework for the study, explaining its importance and relevance. The objectives of this project are presented below.

The following six chapters refer to the theoretical foundation, including fundamental concepts of the myocardium and the problems adjacent to it, SPECT exams, more specifically MPS, anxiety as a neurological problem and musical interventions in medical imaging exams, concepts that are consider relevant to understanding the project theme. The eighth chapter specifies the materials and methods applied within the scope of the study.

Chapter nine presents all the results divided into three phases: Cross-sectional study (CSS), Pilot study (PS) and comparison of the two studies. These results are then discussed in chapter ten. The conclusions of this study are found in chapter eleven.

Finally, the limitations of the study and future perspectives of the work developed are presented in chapter twelve. In chapter thirteen the references used are described and the study publications and communications are listed in chapter fourteen.

# 2. The Myocardium

## 2.1 Structure and functions

The heart is a muscular primary organ of our circulatory system, and its muscle tissue is known as myocardium, from the Greek (*myos* = muscle) + (*kardio* = heart) (28) (Figure 1).

The heart contains four main sections (chambers) made of muscle and is divided into three layers (7,29):

- Pericardium: It encloses the heart, and it contains an outer fibrous sac, and an inner layer of mesothelial cells that provide both lubrication and connective tissue containing fat, arteries, veins, and nerves. The pericardium prevents the heart from expanding excessively due to overfilling with blood.
- Myocardium: The muscle layer.
- Endocardium The inner layer of the heart in between the muscle and the heart cavities. It contains connective tissue and an inner layer made of endothelial cells.

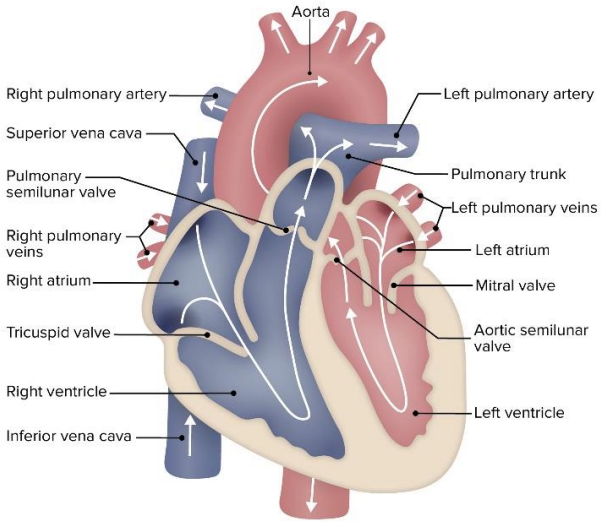



**Figure 1:** General structure and flow of blood through the heart

(Adapted from: (30))

The brain and nervous system direct your heart's function and the heart pumps blood throughout your body. Embedded in the myocardium there is a specialized conducting tissue capable of generating a coordinated heart rate. The electrical impulse is initiated by a group of pacemaker cells, located in the sinoatrial node in the right atrium. These cells can generate spontaneous electrical impulses, at around 100 beats per minute, although nervous input controls and lowers this value at rest (31–33).

The contraction impulse subsequently reaches the atrioventricular node. The signal is conducted to the ventricles by the atrioventricular bundle and the Purkinje fibers (34,35). The atrioventricular node is in the junction between the atria and the ventricles. The electrical conduction through the atrioventricular node is slow, preventing the ventricles from contracting at the same time as the atria. An atrial contraction is thus followed by a ventricular one and it happens around 70 − 80 times per minute in a healthy human at rest (34,36–38). This electrical activity can be recorded on an Electrocardiogram (ECG) (Figure 2).

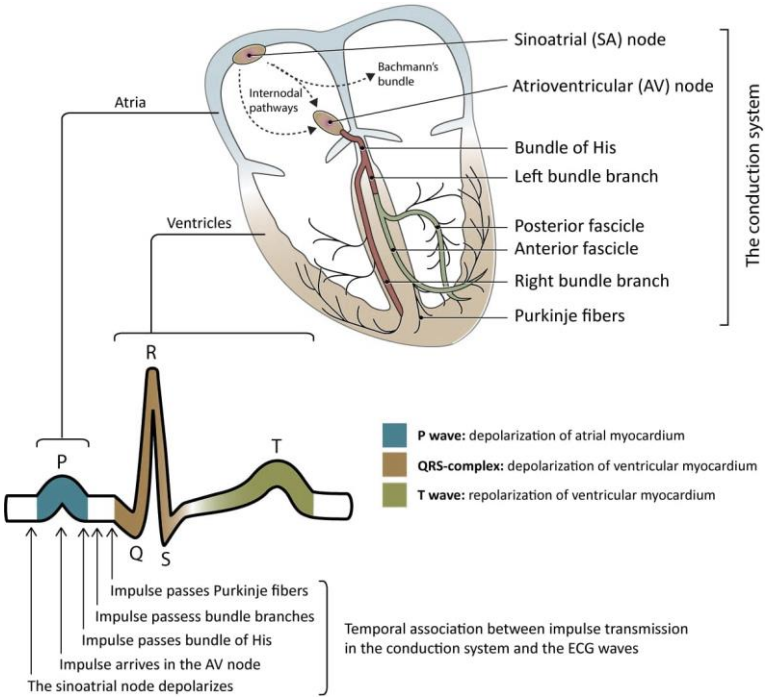



**Figure 2:** The cardiac conduction system and ECG

(Adapted from: (39))

The P wave comes from the depolarization of the atria, pushing the blood into the ventricles through the AV valves. During the QRS complex the atria relax while the ventricles' contraction is triggered, forcing the blood into the arteries through the semilunar valves. The QRS complex corresponds to the depolarization of the ventricles and masks an inverted P wave that would represent the atria repolarization. The magnitude of the QRS complex is much larger than the P wave due to a much more abundant muscle tissue in the ventricles compared to the atria. The T wave represents the repolarization of the ventricles. It is easy to notice that depolarization means contraction while repolarization triggers relaxation. It might seem strange at first that the recorded R and T wave on the ECG have the same polarity, since they represent a depolarization and repolarization respectively, but both events happen in opposite directions, with repolarization starting in the apex of the heart towards the base (Figure 2) (31,34,37,38).

Myocardial Perfusion is the process of delivering the nutrients contained in the arterial blood to the capillary bed of the heart muscle and this is done through the coronary circulation (7,38). The blood flow in the circulatory system is mainly controlled by the heart, more specifically by pressure differences generated by myocardial contraction, working together with the heart valves. The right heart chambers propel unoxygenated blood through the pulmonary circulation, and the left heart propels oxygenated blood through the systemic circulation. The direction of blood flow begins at the left ventricle of the heart, flows to the arteries, then arterioles, capillaries of each body organ, venules, veins, right atrium, right ventricle, pulmonary artery, lung capillaries, pulmonary veins, left atrium and then goes back to the left ventricle (Figure 3) (7,29,38).

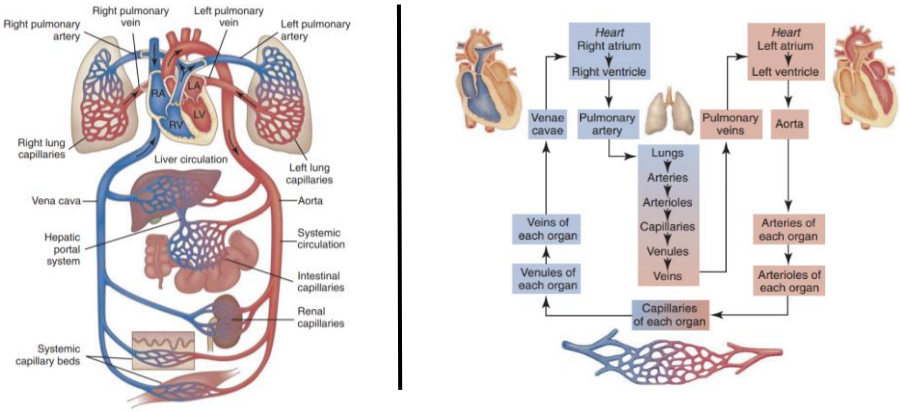



**Figure 3**: Legend: Pulmonary and Systemic Circulatory Systems. Schematic diagram showing serially connected pulmonary and systemic circulatory systems and the direction of blood flow; RA, Right atrium; RV, right ventricle; LA, left atrium, LV, left ventricle.

(Adapted from: (29))

## 2.2 Vital signs

Vital signs measure the body's basic functions. Measuring vital signs is usually the first step in almost every medical evaluation and its useful to detecting or monitoring health issues and alerting medical professionals to potential concerns such as heart diseases (40,41). The most used vital signs for medical measurements are the blood pressure, heart rate, respiratory rate, blood oxygen levels and body temperature (40,42).

### 2.2.1 Blood Pressure

When the heart beats, blood pulses through the arteries to travel throughout the body. The pulse of the blood flow and the pressure it exerts change from moment to moment.

Blood pressure is measured in millimetres of mercury (mmHg) and is given as 2 measurements, systolic pressure, and diastolic pressure. Systolic blood pressure is the higher number and refers to the amount of pressure experienced by the arteries while the heart is beating. Diastolic blood pressure is the lower number and refers to the amount of pressure in the arteries while the heart is resting in between heartbeats. Medical professionals measure blood pressure using these numbers because it is a standard way of describing the force of the pulsing blood (43,44). Both numbers give important information about patients' health. However, the healthcare provider might give more importance to high systolic pressure to determine the risk of heart disease (44). There are five stages of blood pressure, presented in Table 1.

**Table 1**: Stages of Blood Pressure

(Adapted from: (45))

| **Hypertension disease staging** | **Other risk factors, HMOD, or disease** | BP (mmHg) grading | | | |
|---|---|---|---|---|---|
| | | High normal SBP 130–139 DBP 85–89 | Grade 1 SBP 140–159 DBP 90–99 | Grade 2 SBP 160–179 DBP 100–109 | Grade 3 SBP ≥180 or DBP ≥110 |
| Stage 1 (uncomplicated) | No other risk factors | Low risk | Low risk | Moderate risk | High risk |
| | 1 or 2 risk factors | Low risk | Moderate risk | Moderate to high risk | High risk |
| | ≥3 risk factors | Low to moderate risk | Moderate to high risk | High risk | High risk |
| Stage 2 (asymptomatic disease) | HMOD, CKD grade 3, or diabetes mellitus without organ damage | Moderate to high risk | High risk | High risk | High to very high risk |
| Stage 3 (established disease) | Established CVD, CKD grade ≥4, or diabetes mellitus with organ damage | Very high risk | Very high risk | Very high risk | Very high risk |

### 2.2.2 Heart rate

The heart rate (HR), also known as pulse rate, is the number of times the heart beats per minute. The age and general health affect the HR and so, pulse can modify from person to person (46). The following Table 2 shows the normal HR values, depending on age for adults.

**Table 2**: Average heart rates for different ages and genres

(Adapted from: (47))

| Age | Men | Women |
|---|---|---|
| 18-25 | 62-73 bpm | 64-80 bpm |
| 26-35 | 62-73 bpm | 64-81 bpm |
| 36-45 | 63-75 bpm | 65-82 bpm |
| 46-55 | 64-76 bpm | 66-83 bpm |
| 56-65 | 62-75 bpm | 64-82 bpm |
| Over 65 | 62-73 bpm | 64-81 bpm |

### 2.2.3 Respiratory rate

The respiratory rate (RR), or breathing rate, is the number of breaths that a person take per minute. It is one of the main vital signs, along with blood pressure, heart rate, and temperature. When a person inhales, oxygen enters their lungs and travels to the organs. When they exhale, carbon dioxide leaves the body (48,49). A normal respiratory rate plays a critical role in keeping the balance of oxygen and carbon dioxide even in the body. Many factors, including age and activity levels, affect a person's respiratory rate. Adults usually take between 12 and 20 breaths per minute. Although the normal RR can vary slightly between individuals, there is a range that medical professionals usual used (48).

The following Table 3 shows the normal RR values, depending on age:

**Table 3:** Normal RR values in different ages

(Adapted from: (48))

| Group Age | Age | Normal Respiratory Rate |
|---|---|---|
| **1** | 1 year | 30 to 40 |
| **2** | 2 – 5 years | 20 to 40 |
| **3** | 6 – 10 years | 15 to 25 |
| **4** | 11 - 18 years | 15 to 20 |
| **5** | 18 – 70 years | 12 to 20 |
| **4** | >70 years | 15 to 20 |

### 2.2.4 Blood Oxygen Levels

$SpO_2$, also known as oxygen saturation, is a measure of the amount of oxygen-carrying hemoglobin in the blood relative to the amount of hemoglobin not carrying oxygen. The body needs there to be a certain level of oxygen in the blood, or it will not function as efficiently. In fact, very low levels of $SpO_2$ can result in very serious symptoms (Chan et al., 2013). This condition is known as hypoxemia. There is a visible effect on the skin, known as cyanosis due to the blue (cyan) tint it takes on. Hypoxemia (low levels of oxygen in the blood) can turn into hypoxia (low levels of oxygen in the tissue). A typical, healthy reading is 95–100%. (Chan et al., 2013; Jubran, 2015). The following Table 4 shows the blood oxygen levels in different stages:

**Table 4**: Gradation of oxygen saturation levels

(Adapted from: (50))

| Blood oxygen saturation ($SpO_2$) | | |
|---|---|---|
| 100 - 98 % | | Normal |
| 97 - 95 % | | Insufficient<br>Tolerable, patient hardly notices any influence |
| 94 - 90 % | | Decreased<br>Immediate intervention (eating, exercise) |
| < 90 % | | Critical<br>Referral to specialist |
| < 80 % | | Severe hypoxia<br>Hospitalization |
| < 70 % | | Acute danger to life |

# 3. Cardiovascular diseases

## 3.1 Background

CVDs are a group of disorders of the heart and blood vessels, and the leading cause of morbidity and mortality worldwide, affecting millions of people every year, estimated 17.9 million lives each year (1–3).

A wide array of problems can arise within the cardiovascular system, a few of which include endocarditis, rheumatic heart disease, and conduction system abnormalities (1,51,52). CVDs, refers to cerebrovascular diseases, peripheral artery diseases (PADs), aortic atherosclerosis and coronary artery diseases (CADs) which is also referred to as coronary heart diseases (CHDs). (2,51).

## 3.2 Coronary Heart Disease

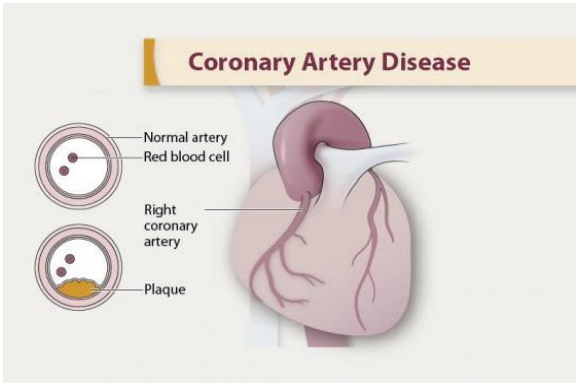
CAD is the most common form of CVD, accounting for more than 40% of deaths in Europe (4) (53). In CAD, the patient's arteries become narrowed by atheromatous plaques or thrombus formation, due to cholesterol plaque formations. (6,7).

Atherosclerosis (lipoprotein-driven disease) is the most common form of coronary artery disease (7). It causes plaque formation at specific sites of the arterial tree through intimal inflammation, necrosis, fibrosis, and calcification. The plaque obstruction leads to impairment in blood flow, decreasing oxygen supply to the myocardium (2,6) (Figure 4).

CAD is a multifactorial phenomenon, influenced by e.g., etiologic factors, categorized into non-modifiable and modifiable factors. Non-modifiable factors include gender, age, family history, and genetics. Modifiable risk factors include smoking, obesity, lipid levels, and psychosocial variables (16).

The patients in this common heart condition may experience chest discomfort (angina), which is a classic symptom that indicates a lack of blood and oxygen supply to the cardiac tissues (ischemia). Myocardial infarction is caused by prolonged ischemia, leading to irreversible damage (necrosis) to the myocardium (7,52,54).

A)




B)


**Figure 4:** Coronary artery disease: normal artery and artery obstructed by cholesterol plaque B) Atherosclerosis stages in coronary artery disease, caused by cholesterol plaque.

(Adapted from: (55))

Vital signs measure the body's basic functions. Measuring vital signs is usually the first step in almost every medical evaluation and its useful to detecting or monitoring health issues and alerting medical professionals to potential concerns such as heart diseases (40,41). The most used vital signs for medical measurements are the blood pressure, heart rate, respiratory rate, blood oxygen levels and temperature (40,42).

## 3.3 Cardiovascular risk factors and medication

Cardiovascular diseases are associated with a set of factors that are called risk factors. The cardiovascular risk factors (CVRFs) contribute to the development of many cardiovascular diseases (56–58). There are two types of CVRFs:

- Modifiable: Factors that can be modified and corrected, such as High Blood Pressure (HBP), Diabetes Mellitus (DM), Dyslipidemia (high cholesterol and triglyceride levels), obesity, smoking, sedentary lifestyle, excessive stress, and excessive consumption of alcohol.

- Non-modifiable: Factors that cannot be changed, such as age, gender, and family history of heart disease.

Medical diagnostics are essential to assess the risk of developing cardiovascular disease. The earlier the diagnosis, the greater the chances of preventing the increase of CVD (58).

After diagnosing patients with heart disease, medical professionals prescribe medications to treat heart failure, some of which must be taken for the rest of their lives (59,60). The type of medicine used depends on the type of disease, and these can be divided into different categories (59–61):

- Anticoagulants: Decreases the clotting (coagulating) ability of the blood. Sometimes called blood thinners, although they do not actually thin the blood. This medication helps to prevent harmful clots from forming in the blood vessels, may prevent the clots from becoming larger and causing more serious problems and they are often prescribed to prevent first or recurrent stroke (62);

- Antiplatelet Agents: Keeps blood clots from forming by preventing blood platelets from sticking together. Normally helps the patients. Helps prevent clotting in patients who have had a heart attack, unstable angina, ischemic strokes, TIA (transient ischemic attacks) and other forms of cardiovascular disease (63);

- Angiotensin-converting enzyme (ACE) Inhibitors: ACE inhibitors are medicines that help relax the veins and arteries to lower blood pressure. ACE inhibitors prevent an enzyme in the body from making angiotensin II, a substance that narrows blood vessels. This narrowing can cause high blood pressure and forces the heart to work harder. Angiotensin 2 also releases hormones that raise blood pressure (59,60);

- Angiotensin Receptor Blockers (ARBs): Rather than lowering levels of angiotensin II (as ACE inhibitors do) angiotensin II receptor blockers prevent this chemical from having any effect on the heart and blood vessels. This keeps blood pressure from rising (59,60);

- Angiotensin Receptor-Neprilysin Inhibitors (ARNIs): Neprilysin is an enzyme that breaks down natural substances in the body that open narrowed arteries. By limiting the effect of neprilysin, it increases the effects of these substances and improves artery opening and blood flow, reduces sodium (salt) retention, and decreases strain on the heart (64);

- Beta Blockers (BB): Decreases the heart rate and force of contraction, which lowers blood pressure and makes the heart rate slowly and with less force. Normally used to lower blood pressure, for cardiac arrhythmias (abnormal heart rhythms), treat chest pain (angina) and prevent future heart attacks in patients who had a heart attack (59,60);

- Calcium Channel Blockers (CCB): Interrupts the movement of calcium into the cells of the heart and blood vessels and may decrease the heart's pumping strength and relax blood vessels (65);

- Cholesterol-lowering medications: reduce high blood cholesterol levels, specifically, lower Low-density lipoprotein (LDL) cholesterol also known as ("bad") cholesterol. This lipid lowering drugs are a primary prevention of coronary heart disease (66);

- Digitalis Preparations: Increases the force of the heart's contractions. Can be beneficial in treating heart failure and irregular heart rate, especially when the patient isn't responding to other standard treatments including ACE inhibitors, ARBs, and diuretics guide (67);

- Diuretics: Causes the body to rid itself of excess fluids and sodium through urination. Helps to reduce the heart's workload. Also decreases the buildup of fluid in the lungs and other parts of the body, such as the ankles and legs. Different diuretics remove fluid at varied rates and through different methods (59).

# 4. Medical Imaging Procedures

## 4.1 Background

Medical imaging refers to the techniques and processes used to create images of the human body (or parts thereof) in medical and NM services (68,69). In recent years medical imaging procedures have become an asset to modern medicine. The use of imaging techniques has experienced rapid growth with a sustained rise in the utilization of imaging examinations for various medical conditions across multiple subspecialties to study anatomy and functions of human body (70–73). The wide range of imaging techniques available today enables healthcare professionals to make informed clinical decisions, monitor patients' health, and accurately diagnose illnesses (70–73).

Emission tomography is a medical imaging modality, belonging to NM, which essentially includes Positron emission tomography scan (PET) and SPECT (74,75). Both techniques use radiopharmaceuticals to obtain images with information about the physiological properties of the body (75). Clinical Emission tomography (ET) imaging procedures involve many main steps (Figure 5). After the production of the radiopharmaceutical, it is administered to the patient, normally by injection, and following a waiting period (depending on the pharmacokinetics of the radiopharmaceutical and the objective of the study in question), the patient proceeds to image acquisition.

Data acquisition takes place while the patient lies still on a bed. The radioactive isotope with which the radiopharmaceutical was labelled emits gamma rays due to radioactive decay and, as this radiation is emitted, the gamma rays are detected by the system, which rotates around the patient, acquiring images of the distribution of the radiopharmaceutical in different angles (75,76).

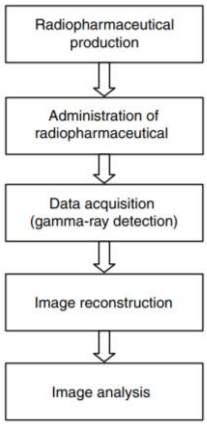



**Figure 5:** Key steps in an emission tomography study

(Adapted from: (76))

From the two major methods in ET, PET and SPECT differ essentially in the type of emission and, consequently, in the way radioactive decay is detected.

While PET uses a positron-emitting isotope, SPECT studies use radiopharmaceuticals labelled with a single photon emitter (75,76), to e.g. diagnose and track the progression of heart disease, such as blocked coronary arteries.

Since this dissertation is directly related to the MPS imaging method, this chapter will be in more detail for the SPECT technic.

In PET scans a positron (particle with roughly the same mass as an electron but oppositely charged) react with electrons in the body and when these two particles combine, they annihilate each other. This annihilation produces a small amount of energy in the form of two photons that shoot off in opposite directions (about 180$^{o}$). The detectors in the PET scanner measure these photons and use this information to create images of internal organs.

## 4.2 SPECT

### 4.2.1 Background

In SPECT method the acquiring tomographic studies allows obtained 3D images of the distribution of a radiopharmaceutical, being sensitive for the evaluation and quantification of processes physiological aspects of the organism (77). This technique involves acquiring planar views of the patient, from different directions, allowing a high number of projections and, consequently, through reconstruction algorithms, it is possible to obtain a three-dimensional image of sections of the human body. Conventionally, these images include three orthogonal planes – axial, sagittal, and coronal. Normally the axial images are obtained directly from the SPECT acquisition and the remaining planes are obtained from a set of axial cuts (78).

In a myocardial SPECT study, once transaxial data are available and considering cardiac positioning (i.e., the heart sits obliquely in the mediastinum) (79), the cardiac volume is realigned to present a standard orthogonal heart orientation for its main axes. Therefore, the planes in SPECT or Gated-SPECT studies of the myocardium have a different designation: horizontal long axis, HLA (transaxial plane), vertical long axis, VLA (sagittal plane) and short axis, SA (coronal plane) (Figure 6) (10).

Horizontal long-axis (transaxial) views allow visualization of the lateral wall, septum, and apex of the LV myocardium, as well as the free wall of the right ventricle. Vertical long axis views allow visualization of the anterior wall, apex, and inferior wall of the LV myocardium. The short axis allows imaging of the anterior, lateral, inferior wall and septum of the LV myocardium (Figure 6) (10,79).

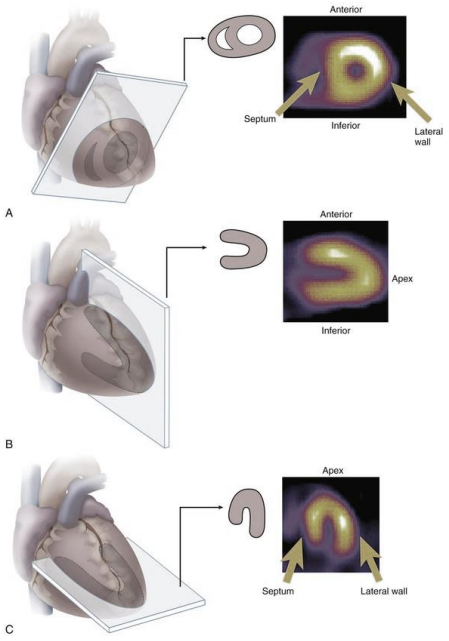



**Figure 6**: Standard SPECT imaging display. A - The short-axis images represent a portion of the anterior, lateral, inferior, and septal walls. B - Vertical long-axis images represent the anterior wall, apex, and inferior wall. C - Horizontal long-axis images represent the septum, apex, and lateral walls.

(Adapted from: (78))

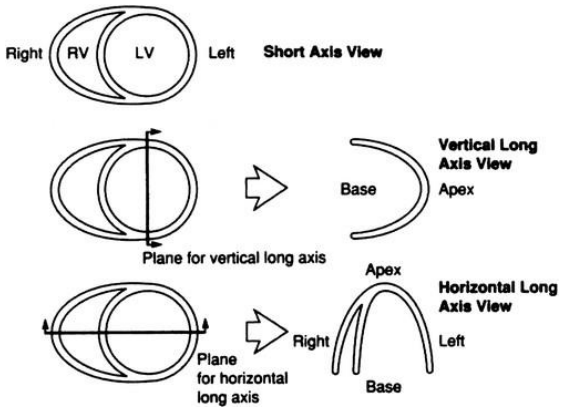



**Figure 7**: Cardiac plane definition and display for tomographic imaging modalities

(Adapted from: (80))

To obtain SPECT images, it is necessary to acquire a complete set of images around the patient, without any temporal modification. The axis is then well defined (parallel to the patient's longitudinal axis) and there is a rotation of the gantry and the tomographic projections are acquired according to a rotation arc of 360° of the detector for tomographic studies of the body and 180° for cardiac studies (79,81,82).

The simplest configuration of a SPECT system involves only one detector, but by increasing the number of detectors, it is possible to improve the sensitivity of the system, as this is approximately proportional to the number of detectors (81). Subsequent data acquisition can be carried out in two ways, which differ in the configuration of the movement of the detectors: continuous acquisition (where data is acquired as the detectors rotate continuously around the patient) or step by step (gradual, also referred to as such as step and shoot), where the detector stops at different angular positions during the period in which data is collected, this being preferable in cases of unstable patients (83,84).

### 4.2.2 Gamma Cameras

Gamma Camera (GC) detects and allows determining the spatial origin of gamma rays emitted by radiopharmaceuticals administered to the patient, allowing an image to be obtained, through which it is possible to distinguish the areas of highest and lowest concentration of gamma rays, called “hot spots” and “cold spots” (85) (Figure 8). This type of equipment consists in a set of planar detectors, normally two coupled to a gantry, allowing the simultaneous acquisition of different plans of a specific region of the human body. In CG, besides the electronic mechanism, each detector consists in a collimator, a scintillation crystal, a light guide, and photomultiplier tubes (75,76,86) (Figure 9).

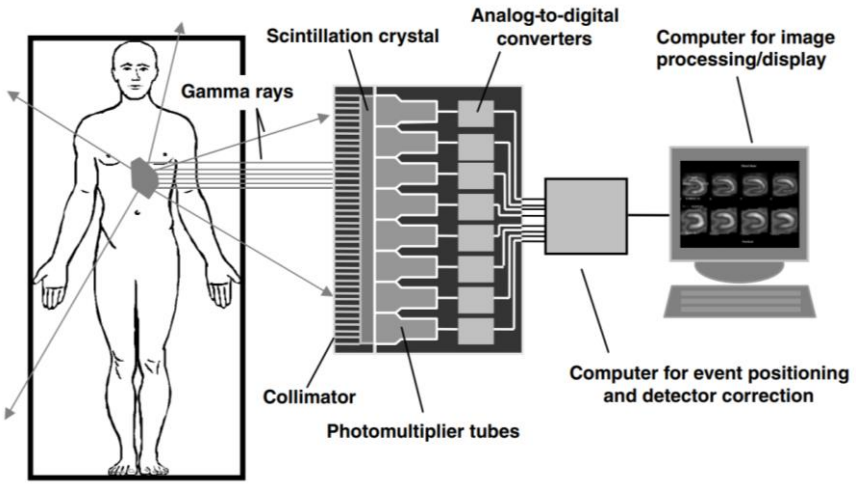



**Figure 8:** Schematic diagram of a conventional gamma camera used in SPECT.

(Adapted from:(75))

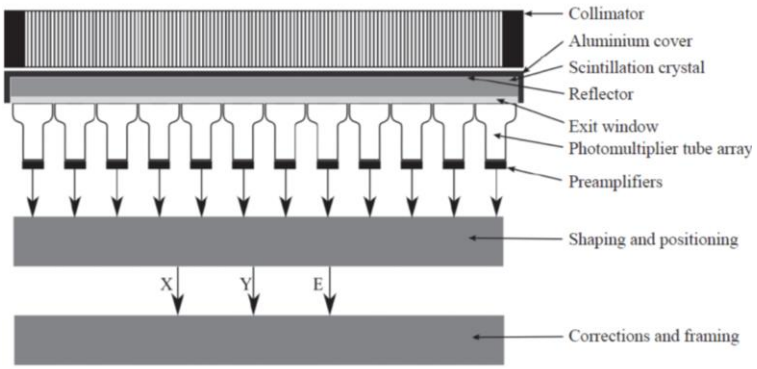



**Figure 9:** Schematic of a gamma camera detector

(Adapted from: (87))

A collimator consists of a plate made of lead, composed of a network of holes, in order to allow photons coming from a certain direction to pass to the scintillator crystal, with all remaining photons being absorbed by the lead walls (septal walls) constituents of the holes. When interacting with the scintillation crystal, usually sodium iodide activated with thallium (NaI (Tl) photons are absorbed, releasing an electron that passes through the crystal (79,88). Then the scintillation phenomenon occurs, when the electrons release their energy into photons in the visible light spectrum. The scintillation crystal converts high-energy photons into low-energy photons, which are then forwarded through the light guide to the Photomultiplier Tubes (PMTs), which allow the light signal (incoming photons) to be converted into an electrical signal (photoelectrons) with measurable magnitude, enabling the quantification of the energy deposited (89,90). Each PMT, a vacuum-sealed glass tube, contains an entrance window, photocathode, focusing electrode, dynodes (electron multipliers) and anode. Thus, the light emitted by the scintillation crystal, when passing through the input window, will excite the electrons in the photocathode, emitting photoelectrons, which are accelerated and focused by the focusing electrode in the first dynode, where they multiply repeatedly in the successive dynodes, due to the secondary electron emission. After passing through the last dynode, the electrons generated there are collected by the anode, generating an electrical signal at the output of the PMT (85,87,89) (Figure 10).

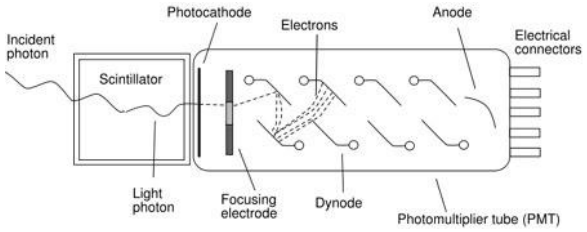



**Figure 10:** Schematic diagram of a scintillation detector comprising a scintillation material coupled to a photomultiplier tube.

(Adapted from: (91))

The photomultiplier tubes are coupled to the scintillation crystal, allowing the interaction of each gamma photon in the crystal causes each PMT to register a given intensity of light.

The energy deposited by the photon incident on the crystal is proportional to the sum of all output pulses from the PMTs, which has 3 components: X and Y (spatial location of the scintillation) and Z (energy deposited in the crystal by gamma ray). Considering the collimation process, it is expected that only the photons emitted perpendicular to the GC field of view interact with the crystal, however during SPECT, there is interaction of photons with the medium, which can suffer Compton dispersion and loss of energy, which leads to a change in the trajectory of the photons, not reflecting their origin (85,89).

It is necessary discriminate the photons incident on the crystal, which is possible by passing the Z signal through a Pulse-Height Analyzer (PHA). The PHA checks whether the photon energy is within the energy range acceptable, defined by the characteristic energy window of each radionuclide. In the final image it´s only consider the Z pulses of events occurring with an energy within of the considered window, registering the detection of a gamma ray, with its location being determined by the X and Y components of the signal (82,86,89).

In a selection of a radiation detector for NM imaging, it is necessary to consider several properties such as (78,89):

- Detection Efficiency – Sensitivity (rate of events recorded per unit of time and per unit of radioactive concentration);
- Dead Time Response - Temporal Resolution (time required for the detector to process an individual event);
- Spatial Resolution (the number of picture element (pixel) used in construction of the image)
- Energy Resolution (measure of the detector's ability to distinguish two very close energy values)

### 4.2.3 Radiopharmaceuticals

Radiopharmaceuticals, fundamental basis of NM, are chemical substances labelled with radioactive isotopes that have physiological or biochemical affinity with structures of the human body, since the physiological vector corresponds to an organic molecule recognized by the organism, as it is like some substance processed by the organ or tissue that is intended to be studied.(10,92). The radionuclide is responsible for the emission radiation resulting from the radioactive decay process, in the case of SPECT, in the form of gamma radiation. A part of this radiation will be detected outside the body and will allow images to be obtained of organs or tissues (75,79,92).

Radiopharmaceuticals can be classified into perfusion radiopharmaceuticals and specific radiopharmaceuticals. Perfusion radiopharmaceuticals are transported through the bloodstream to the target organ, while specific radiopharmaceuticals contain a biologically active molecule, with the purpose to bind to cellular receptors (93).

The radiopharmaceutical to be administered depends on the study to be carried out and certain characteristics must be considered. The half-life time ($T_{1/2}$) is defined as the time after which the activity of the isotope becomes half of the value of the initial activity (94,95). Ideally, the value should be as small as possible, but still enable the correct biodistribution of the radiopharmaceutical and a quantity of detected photons favourable to minimizing image acquisition time, without compromising the minimum clinical image quality (93,96) The energy and type of emission are also factors to consider, and radionuclides that emit α or β particles should not be used for diagnostic purposes, due to the damage they cause to biological tissues (95,97). Therefore, the radiopharmaceutical must contain radionuclides that emit γ rays, with energies between 50 and 300 keV, since below 50 keV γ rays are absorbed by the tissue and are not detected, and above 300 keV effective collimation may not be possible. achieved with traditional collimators, compromising image quality (78,94,96).

Radionuclides used in Nuclear Medicine for diagnosis and therapy are artificially produced in reactors or particle accelerators. They may also be accessible through radioisotope generators (82,92).

There are several radiopharmaceuticals that emit γ radiation. Table 5 shows the more commonly used radionuclides in SPECT studies.

**Table 5:** Radionuclides used in SPECT imaging. min, minutes; h, hours; d, days; y, years. EC, electron capture; IT, isomeric transition; b+, positron decay; β, beta decay

(Adapted from: (98))

| | | | | Energy[a] (keV) | | |
|---|---|---|---|---|---|---|
| | Radionuclides | $T_{1/2}$ | Decay | $E_{\beta^+}$ | $E_{\beta^-}$ | $E_\gamma$ |
| SPECT | $^{99m}$Tc | 6.01 h | IT, $\beta^-$ | | | 140 |
| | $^{123}$I | 13.22 h | EC, $\beta^+$ | | | 159 |
| | $^{111}$In | 2.80 d | EC | | | 245 |
| | $^{131}$I | 8.02 d | $\beta^-$ | | 606 | 364 |
| | $^{125}$I | 59.40 d | EC | | | 27 |

Most of radiopharmaceuticals used in conventional nuclear medicine are compounds labelled with $^{99m}$Tc, since GC are optimized for the physical properties of this radionuclide and it can bind to different substrates or ligands, through a complexation reaction, creating radiopharmaceuticals with affinity for different organs, systems, or receptors in the body (10,92).

The $^{99m}$Tc radionuclide is obtained from the radioactive decay of another radionuclide, molybdenum-99 (parent radionuclide), and can be easily made available, in the hospital environment, from $^{99}$Mo-$^{99m}$Tc generators (92,99). Approximately 87.5% of the $^{99}$Mo atoms in a sample disintegrate through the emission of $\beta^-$ radiation and origin $^{99m}$Tc (92).

The half-life time of $^{99m}$Tc, approximately 6,02 hours, is sufficient for the entire process inherent to its preparation, administration, and acquisition of the SPECT image, and simultaneously allows the patient's exposure time to radiation to be minimized (92,93). $^{99m}$Tc emits γ photons with adequate energy for detection (140 keV), which are easily collimated and allow obtaining images with better spatial resolution (82,92,93,96).

Therefore, 99mTc was the radionuclide most used in this MPS study.

## 4.3 Myocardial Perfusion Scintigraphy

### 4.3.1 Background

Myocardial perfusion scintigraphy (MPS) is a well-established imaging method, using a Single Photon Emission Computed Tomography (SPECT) and is synchronized with the electrocardiogram (Gated) signal (3,4,10). MPS was developed in the 1970s and has been used increasingly in clinical cardiology since the 1980s (3).

Myocardial perfusion scintigraphy, also known as myocardial perfusion imaging or nuclear stress test, is a non-invasive diagnostic imaging technique frequently used to evaluate blood flow to the heart muscle (myocardium) and stratify the risk of CAD (3,4,16,33).

### 4.3.2 Patient Preparation

To these procedures, certain factors or conditions may interfere with or affect the results of the exams. Therefore, the participants need to fulfil some pre-requisites to perform this exam. Patients should have nothing orally (expect oral medication or minimum amount of water), depending in the exam beginning time, to prevent gut activity that may interfere with the evaluation of the inferior wall of the left ventricle (LV), vomiting and possible aspiration, particularly in the context of pharmacological overload tests, particularly with vasodilating agents (e.g. adenosine). Patients should wear comfortable clothing for the exercise portion of the exam. Medications that contain methylxanthines or caffeine and food beverage with caffeine must be avoided for 12-24 hours if vasodilator stress test is anticipated. Before imaging, metal or other potential attenuators must be removed if they project on the imaging field to avoid attenuation artifacts. If the study is being done for primary diagnosis of CAD, certain cardiac medication such as nitroglycerine or β-blockers must be avoided to increase the sensitivity (33,54).

### 4.3.3 Protocol

This medical procedure employs technetium radiopharmaceuticals. The exam can be execute in 1 or 2 days, and both phases can be carried out in first or second (3,4) (Figure 11).

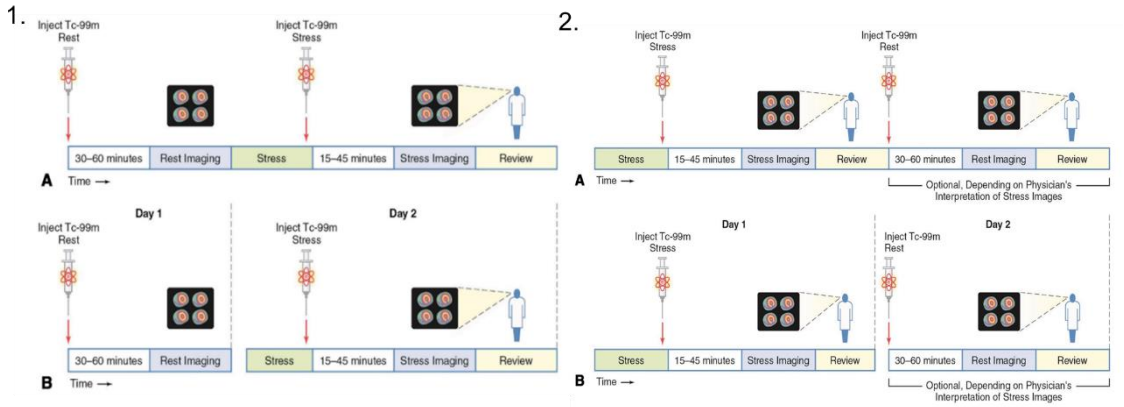



**Figure 11**: One (A) and two (B) day rest-stress Tc99m imaging protocols.

(Adapted from: (4))

In rest phase, the relative regional distribution of perfusion can be assessed The radiopharmaceutical is administered to the patient and after an approximately 30-60 minutes, the SPECT images are obtained (100).

In the stress phase, the patient is asked to walk or run on a treadmill to study the physiological significance of coronary artery diseases by inducing heterogeneity in coronary flow. Resting coronary flow is maintained until there is an approximately 90% reduction of coronary arterial flow. However, the ability to maintain the maximum flow (termed coronary flow reserve) is impaired with approximately 50% coronary stenosis. An increase in coronary flow can be achieved by increased oxygen demand with exercise (treadmill or bicycle), β-adrenergic agonist (dobutamine) or by direct vasodilator (adenosine, dipyridamole) (33,101). The patient's heart rate and blood pressure are monitored during this phase of the test. The diagnostic accuracy of the examination is dependent on the ability of the patient to exercise to induce maximum vasodilatation, a frequently used index, to determine if the patient has exercised adequately to attain his target heart rate (THR). THR is 85% of the predicted maximum heart rate (PMHR) where PMHR=(220-age) beat per minute (33). This is followed by an intravenous injection of radiopharmaceutical. After approximately 30-60 minutes, the SPECT images are obtained (101,102).

Currently, most stress MPS is performed with ECG-gated SPECT for evaluation of both myocardial perfusion and cardiac function simultaneously.

Several stress techniques are available, but the exercise stress test is the preferred method because it provides several important prognostic data in addition to increasing coronary blood flow (33,101,103).

The three most used radiopharmaceuticals for myocardial perfusion scintigraphy (MPS) are thallium-201 ($^{201}$Tl) and the technetium-99m ($^{99m}$Tc) labelled compounds, sestamibi and tetrofosmin (33,82,99).

According to the previous information, depending on the clinical question, the imaging protocol may be a one-day stress-only study (one acquisition) or either a two-day imaging protocol or a one-day stress-first protocol (each involving two acquisitions) (104). Moreover, if a patient has a large body size, this can result in a higher proportion of emitted photons being attenuated within the patient. Therefore, clinical MPS protocols should consider the patient's weight or body mass index (BMI) (101,105).

The optimal amount of activity to be administered to the single patient is determined taking the above variables into account. Therefore, the recommended activities, per single scan, according to the American Society of Nuclear Cardiology (ASNC), may range from 148 MBq (stress-only protocol, BMI = 25 kg/m$^2$) to 1332 MBq (second injection in a one-day stress/rest protocol, BMI = 35 kg/m$^2$), resulting in effective doses of between 1.0 mSv and 10.5 mSv (4). When rest and stress studies are both performed on the same day, the MPS dose may be as low as 4.5 mSv in subjects with BMI = 25 kg/m$^2$, providing the recommended procedure is followed and new technologies are employed. Conversely, the patient dose can reach 13.5 mSv in subjects with a BMI = 35 kg/m$^2$ (4).

Before the images are interpreted, the data should be reviewed for artifacts due to attenuation or zones of unexpected increased activity that may alter the appearance of the myocardium. In the absence of artifacts, the images are evaluated for areas of decreased radiopharmaceutical concentration in the stress or rest images and for changes in regional count density when gated data are recorded (100,103,106). Zones of myocardium with tracer concentration below normal with injection at rest are usually associated with myocardial scar, but fixed defects with uptake greater than 50% of normal regions are often viable. Defects seen at stress that improve on the resting study are usually due to ischemia. (33,100).

The examination report for each patient must contain the information about the condition of the patient at the time of injection should include the type of stress (e.g., treadmill or bicycle), exercise level achieved, heart rate, blood pressure, symptoms, brief description of the electrocardiogram at rest, and changes induced by stress. The duration of exercise should also be stated. With pharmacologic stress, the type, dose of drug, duration of infusion and symptoms, heart rate, blood pressure, and ECG changes should be noted. Information about the distribution of radiopharmaceutical should include the site and extent of reduced perfusion, the likely coronary vessel involved, and the relationship of regional wall thickening to abnormal perfusion (3,16,54,100).

# 5. Image Quality

## 5.1 Image quality characterization

Image quality can be defined as the attribute of the image that influences the clinician's certainty to perceive the appropriate diagnostic features from the image visually. Specifically in NM, image quality is related to the GC's ability to distinguish anatomical structures with differences in radiopharmaceutical uptake. Therefore, after the reconstruction process, the aim is to obtain SPECT images representing the 3D distribution of radioactivity in each volume of tissue that are as reliable as possible, to achieve absolute quantification (107,108).

Image quality in NM can be evaluated using objective and subjective methods. Qualitative assessment involves a visual interpretation, for example, to evaluate the spatial resolution of the image, depending on the skill/experience of the observer, as well as the complexity of the human visual system, meaning it is a subjective method of characterizing image quality. On the other hand, quantitative image assessment encompasses objective methods of characterizing its quality, as it is based on measurements of physical characteristics such as, for example, contrast, noise, and spatial resolution (108–110).

The parameters used to characterize image quality are closely related to each other, as the improvement of one often leads to the deterioration of another. In this way, a compromise must be sought between all of them. (111,112).

In this specific study, three fundamental parameters for characterizing SPECT image quality were analysed:

- Spatial Resolution
- Noise
- Contrast

### 5.1.1 Spatial Resolution

Spatial resolution refers to the minimum distance between two-point sources so that they are observed as distinct points in the image (110). The digital medical image is a function f (x, y) in grey level scale partitioned into spatial coordinates that can be represented by a matrix, whose row and column indices identify an image point (pixel) (69,110).

A region of interest (ROI) is a portion of an image that you want to filter or operate on in some way. ROI represents as a binary mask image. In the mask image, pixels that belong to the ROI are set to 1 and pixels outside the ROI are set to 0 (113,114).

The construction of these ROI allows us to identify the number of pixels counts in the interest area and the standard deviation values. With these data it is possible to calculate the image quality parameters highlighted above (10,113).

### 5.1.2 Noise

NM images are subject to statistical noise, arising from the random nature of the radioactive decay process that causes statistical variations in the observed count rate (115). The average percentage of noise in a SPECT image is usually calculated by the ratio between the standard deviation and the average value of the counts in the sample of interest (Equation (1)) and, to be clinically meaningful, the noise in the reconstructed image must be less than 33% (116,117). Noise in the SPECT image depends on several factors, such as acquisition time, number of projections, matrix size, GC sensitivity, radiopharmaceutical, as well as the dose administered (115).

$$\%\ \text{Noise} = \frac{\text{Standard Deviation}_{\text{Myocardium}}}{\text{Average Count}_{\text{Myocardium}}} \times 100 \quad (1)$$

### 5.1.3 Contrast

Contrast is the differences in intensity between different regions of the image, corresponding to different concentrations of activity in the patient's body. Therefore, to obtain better contrast levels, it is essential to use radiopharmaceuticals that result in the highest uptake ratio between the lesion and the background, that is, the highest concentration ratio between them. Thus, the choice of radiopharmaceutical appears to be a relevant factor regarding contrast values, which is why different radiopharmaceuticals are used depending on the objective of the study, as they have their own properties that determine the affinity with certain structures of the body (110,111). Quantitatively, the contrast of a SPECT image can be defined as the ratio between the difference of the average counts from the region of interest with background (Equation (3)). (111,116,117).

$$Contrast = \frac{Average\ Count_{Myocardium} - Average\ Count_{Background}}{Average\ Count_{Background}} \quad (2)$$

## 5.2 Factors that influence image quality

The SPECT image acquisition and formation processes are exposed to several factors, leading to image degradation, which cause distortion of the acquired data and, consequently, impair image quality, as well as absolute quantification (118). The factors that influence image quality can be divided, according to their nature:

- Instrumental factors: Sources of image degradation are related to the performance of detection systems. The uniformity of the GC response and those associated specifically with the detector stand out, such as detection efficiency (sensitivity), spatial resolution, dead time, counts and acquisition orbits. Parameters such as sensitivity and resolution are mostly defined by the collimator, which must be appropriate to the object under study. For example, a high-resolution collimator is suitable for acquiring small structures or those close to organs with high uptake, promoting greater precision. The image quality is also dependent on the size of the selected matrix and the number of projections, which must cover the entire region of interest and must be at least equal to the size of the matrix (117).
- Physiological factors: SPECT images can be affected by factors inherent to the radiopharmaceutical and the patient, given that the dose fraction administered to a given organ and the attenuation suffered by the photons before reaching the detector influence the counting statistics, which is an important factor in the quality and image quantification, representative of the information available. Characteristics relatable with the radiopharmaceutical, such as its specificity, biokinetics and administered dose, are related to the image quality, since the contrast depends on the spatial distribution of radioactivity within the area of interest, and this distribution depends on the rate of uptake, metabolism and physical half-life of the radionuclide (111,117). During acquisition, the patient's movements, whether voluntary or involuntary (cardiac and respiratory), interfere with the quality of the data obtained, degrading the spatial resolution, and causing artifacts such as, for example, the partial volume effect, resulting in blurring of the image object study and the blurring of contours. Regarding the patient, it is necessary to take their anatomy into account, as the size and geometry of organs and tissues influence the dispersion and attenuation of photons (119). Since SPECT image quality is dependent on count statistics, acquisition time is an extremely important parameter, directly related to the patient's tolerance, in order to avoid motion artifacts (10,119).
- Physical factors: Gamma photons, emitted from the patient's body, are subject to various interactions with the surrounding environment, both with the body's own tissues and with GC detectors. This interaction, between the emitted radiation and the matter, can lead the photon to deviate from its initial trajectory or even prevent it from interacting with the scintillation crystal and, consequently, contributing to the formation of the image. The physical phenomena with the greatest influence on SPECT image quality are attenuation, Compton effect and statistical noise resulting from the independent and random processes of radioactive decay and photonic interactions, which leads to inaccuracies in activity estimates (10,116).

For this study, we will evaluate and analyse in more detail the physiological factors inherent to the patient, such as the patients' voluntary and involuntary movements, and the possible causes of these phenomena, such as anxiety, which is described in the next chapter.

# 6. Anxiety

## 6.1 Background

Anxiety, from the Latin "*anxietatis",* is an emotional reaction and generally adaptive feeling that occurs when there is a perceived threat to physical and/or psychological integrity. (120,121).

Anxiety levels out of proportion can be very upsetting, as it leads to psychological reactions, such as apprehension, insecurity, anguish, sense of threat, fear, decreased perception of self-efficacy, discomfort, and irritability. (27,122).

High anxiety levels also result in adverse physiological manifestations such as increased blood pressure, heart rate, blood oxygen, and blood cortisol levels and decreased immune response, enhancing the risk of infection (27,121–124).

## 6.2 Neurobiological mechanisms of anxiety

Anxiety and vigilance states are regulated by neural networks involving multiple brain regions (122,125,126).

The limbic system is a complex set of structures that involves the hippocampus, the amygdala and thalamus (Figure 12) (126–129).

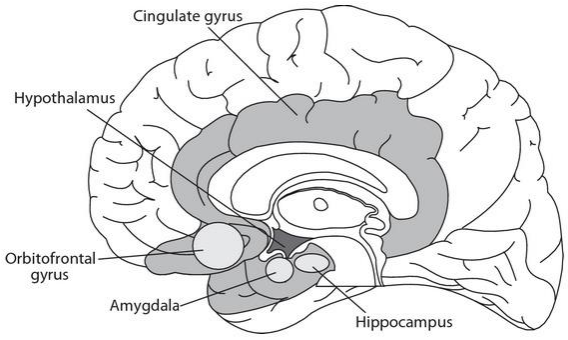



**Figure 12:** Anatomical illustration of important areas of the limbic system

(Adapted from: (126))

The Papez circuit, first proposed by James Papez in 1937, is a major pathway of the limbic system and is believed to be involved in our emotional processing (126,129). According to Papez, thalamus is receiving emotional stimuli and sends it to other parts such as the hypothalamus and the cingulate cortex that provides "emotional experience".

The cingulate cortex sends some information about emotional stimuli to the hippocampus and then to the hypothalamus that is responsible for providing emotional responses (Figure 13) (126,129,130).

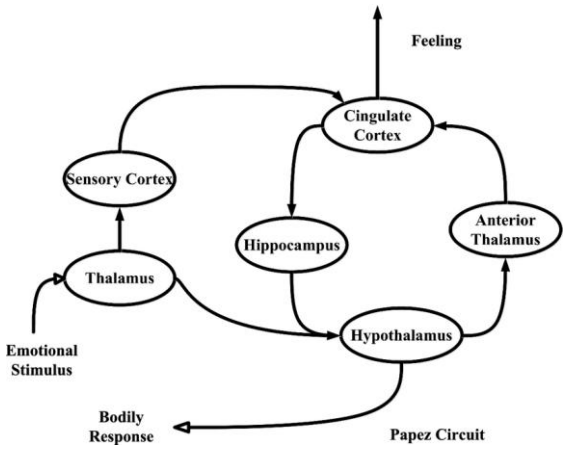



**Figure 13**: The neural structure of the Papez circuit

(Adapted from:(130))

Anxiety is characterized by a variety of neuroendocrine, neurotransmitter, and neuroanatomical disruptions. (122). Nevertheless, being able to functionally identify the most relevant differences is complicated due to the high degree of interconnectivity between the circuits (Figure 14), containing neurotransmitters and neuropeptides in the limbic, brain stem and higher cortical areas of the brain. (122,125,126). In addition, it is also important to consider the neurotransmitters providing communication between these brain regions. Increased activity in emotion-processing brain regions in patients who have anxiety could result from decreased inhibitory signalling by g-amino-butyric-acid (GABA) or increased excitatory neurotransmission by glutamate. (122).

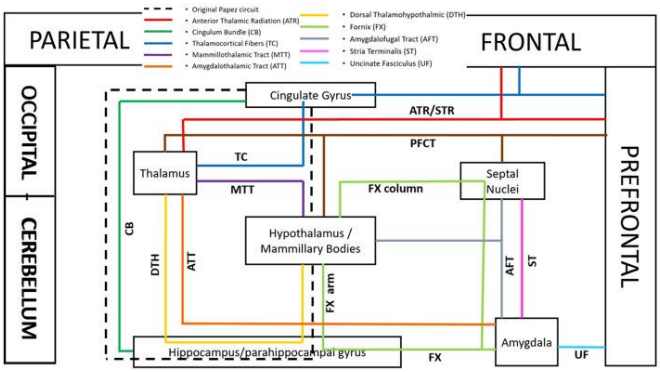



**Figure 14**: Schematic view of the major anterior neuronal circuits (fronto-temporal connectivity) of the limbic system mostly connected to the frontal and temporal cortices.

(Adapted from: (129))

## 6.3 Measurement parameters to anxiety

### 6.3.1 Psychological parameters

STAI:

Different instruments are used to measure anxiety. The STAI is one of the most psychological instrument use diagnose anxiety (131–137). The STAI inventory appeared in 1970 and was authored by Spielberger, Gorsuch and Lushene (138).

Although the STAI was developed at an early stage to assess anxiety in healthy adults, it is increasingly becoming an anxiety measurement tool that can be used in a medical setting and by patients undergoing imaging procedures (120,138–141). STAI is intended to obtain accurate measurements of state and trait anxiety in clinical and research settings.

In STAI, Form Y consists of two twenty-item scales that assess state anxiety and trait anxiety. All items are rated on a 4-point scale (e.g., from “Almost Never” to “Almost Always”). The range of possible scores for form Y of the STAI varies from a minimum score of 20 to a maximum score of 80 on both the STAI-S and STAI-T subscales. STAI scores are commonly classified as “no or low anxiety” (20-37), “moderate anxiety” (38-44), and “high anxiety” (45-80) (142).

According to the patient's needs, the technician carrying out these exams can choose to use only the STAI-S (Form Y-1), with questions only to state anxiety or the STAI-T (Form Y-2) only for trait anxiety (138,143,144). The relationship between self-efficacy and anxiety and general distress in caregivers of people with advanced cancer. (144).The STAI is appropriate for those who have at least a sixth grade reading level.

VAS:

A Visual Analogue Scale (VAS) is one of the pain rating scales used for the first time in 1921 by Hayes and Patterson (145). It is often used in epidemiologic and clinical research to measure the intensity or frequency of various symptoms. For example, the amount of pain that a patient feels ranges across a continuum from none to an extreme amount of pain. From the patient's perspective, this spectrum appears continuous; their pain does not take discrete jumps, as a categorization of none, mild, moderate, and severe would suggest. It was to capture this idea of an underlying continuum that the VAS was devised. (146).

VAS has been widely used in diverse adult populations for example; those with rheumatic diseases, patients with chronic pain, cancer (147), or cases with allergic rhinitis (146).

In addition to rating pain, it has been used to evaluate mood (148) appetite, asthma, dyspepsia, and ambulation (145), and it can be used as a simple, valid, and effective tool to assess disease control (146,147).

VAS normally consists of only 1 item and can be presented in several ways, including (145,146):

- Numerical rating scales: scales with a middle point, graduations, or numbers.
- Curvilinear analogy scales: meter-shaped scales.
- "Box-scales": circles equidistant from each other (one of which the subject must mark).

Although the VAS Scale was created to assess pain, other studies have shown that it can be adapted and is a quick, reliable method for assessing anxiety (149–151).

In this adaptation of the VAS, the scale is also just one-item and classified according to 0 “Nothing anxious” to 10 “Extremely anxious”. VAS scores are commonly classified as “None anxiety” (0), “Mild” (1-4), “Mild-Moderate” (5), “Moderate” (6-7), “Moderate high” (8-9) and “High” (10) (152).

### 6.3.2 Anxiety Biomarker: Cortisol

Cortisol structure

Due to the ambiguity of the diagnosis and many underdiagnosed patients, many studies used laboratory tests that could facilitate the diagnosis of anxiety in clinical practice and would allow for the earliest possible implementation of appropriate treatment. Such potential biomarkers may also be useable in monitoring the efficacy of pharmacological therapy for anxiety disorders (153).

One of the most important steroid hormones with a significant effect on body human metabolism is Cortisol ($C_{21}H_{30}O_5$) (Figure 15) (122,154,155).

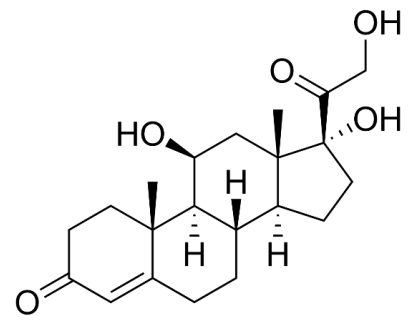



**Figure 15**: Cortisol structure

(Adapted from: (156))

Cortisol is a glucocorticoid produced by the adrenal cortex, mainly in the second half of the night, with the highest levels between 7 a.m. and 8 a.m. During the day, cortisol drops significantly and, in the evening, only about 10% of the morning cortisol remains in the body. Moreover, salivary cortisol differs with age and sex (24).

**Table 6:** Male and female salivary cortisol levels in different age-groups. All means (m) are adjusted for differences in age and proportions are standardized for age. Δ-cortisol - logarithmic morning salivary cortisol – logarithmic evening saliva cortisol. Cortisol concentrations in nmol/L.

(Adapted from: (24))

| | Morning cortisol | | | | Evening cortisol | | | | Δ-cortisol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age-group | n | m | q1–q3 | p | n | m | q1–q3 | p | n | m | q1–q3 | p |
| **Women** | | | | | | | | | | | | |
| 30–39 | 242 | 12.4 | 9.0–17.0 | ref. | 242 | 1.7 | 1.0–3.0 | ref. | 242 | 5.8 | 3.7–10.0 | ref. |
| 40–49 | 291 | 12.4 | 8.0–19.0 | 0.892 | 291 | 2.2 | 2.0–3.0 | 0.834 | 291 | 5.7 | 3.7–9.0 | 0.768 |
| 50–59 | 146 | 11.6 | 8.7–15.2 | 0.260 | 146 | 2.5 | 2.0–3.0 | 0.030 | 146 | 4.7 | 3.0–8.0 | 0.005 |
| 60–69 | 107 | 12.7 | 10.0–18 | 0.732 | 107 | 2.7 | 2.0–4.0 | 0.004 | 107 | 4.7 | 3.3–7.5 | 0.015 |
| ≥ 70 | 52 | 15.1 | 9.2–19.8 | 0.029 | 52 | 3.6 | 2.0–6.0 | <0.001 | 52 | 4.2 | 2.3–9.0 | 0.004 |
| <50 | 533 | 12.4 | 9.0–18.0 | ref. | 533 | 2.2 | 1.4–3.0 | ref. | 533 | 5.8 | 3.7–9.5 | ref. |
| ≥ 50 | 305 | 12.5 | 9.0–18.0 | 0.773 | 305 | 2.7 | 2.0–3.0 | <0.001 | 305 | 4.6 | 3.0–8.0 | <0.001 |
| **Men** | | | | | | | | | | | | |
| 30–39 | 254 | 10.4 | 7.0–15.0 | ref. | 254 | 2.0 | 1.0–3.0 | ref. | 254 | 5.3 | 3.5–9.0 | ref. |
| 40–49 | 276 | 10.6 | 8.0–15.0 | 0.634 | 276 | 2.2 | 2.0–3.0 | 0.101 | 276 | 4.9 | 3.0–8.0 | 0.288 |
| 50–59 | 141 | 11.8 | 8.0–18.0 | 0.030 | 141 | 2.4 | 2.0–3.0 | 0.007 | 141 | 5.0 | 3.0–8.0 | 0.460 |
| 60–69 | 106 | 12.5 | 8.0–17.3 | 0.006 | 106 | 2.5 | 2.0–3.0 | 0.001 | 106 | 4.9 | 3.3–7.6 | 0.459 |
| ≥ 70 | 56 | 12.3 | 9.0–16.8 | 0.049 | 56 | 2.9 | 2.0–4.0 | <0.001 | 56 | 4.2 | 3.0–6.5 | 0.040 |
| <50 | 530 | 10.5 | 7.0–15.0 | ref. | 530 | 2.1 | 1.0–3.0 | ref. | 530 | 5.1 | 3.3–8.5 | ref. |
| ≥ 50 | 303 | 12.1 | 8.0–18.0 | 0.001 | 303 | 2.5 | 2.0–3.0 | <0.001 | 303 | 4.8 | 3.0–7.7 | 0.305 |

When human body is exposed to mental or physical stress, the adrenal glands produce increased amounts of cortisol. (153,154,157). The fluctuations in cortisol secretion activates metabolism, which provides the body with energy (e.g., by releasing glucose into the blood) and changes the conditions of mental reactions (by enhancing the action of other “stress hormones” such as adrenaline and noradrenaline. (154,157–162).

In short, cortisol secretion usually accompanies psychiatric disorders, and its normalization correlates with improvements in the patient's health, showing that can be useful as a biological marker to determine anxiety. (125,154,155,158,161,163).

## Cortisol measurement: ELISA technique

For a quantitative measurement of cortisol, ELISA is a widely used technique (164). ELISA (which stands for enzyme-linked immunosorbent assay) detect the presence of antigens in biological samples. An ELISA, like other types of immunoassays, relies on antibodies to detect a target antigen using highly specific antibody-antigen interactions (165).

There are four major types of ELISA:

- Direct ELISA (antigen-coated plate; screening antibody)
- Indirect ELISA (antigen-coated plate; screening antigen/antibody)
- Sandwich ELISA (antibody-coated plate; screening antigen)
- Competitive ELISA (screening antibody)

The competitive ELISA was the technique we used in this specific study. Therefore, I will only detail this type of ELISA. Competitive ELISAs are commonly used for small molecules, when the protein of interest is too small to efficiently sandwich with two antibodies. They use two specific antibodies, an enzyme-conjugated antibody, and another antibody present in the test serum (if the serum is positive). Combining the two antibodies into the wells will allow for competition for binding to antigens. The presence of a colour change means that the test is negative because the enzyme-conjugated antibody bound the antigens (not the antibodies of the test serum). The absence of colour indicates a positive test and the presence of antibodies in the test serum (Figure 16) (166). The competitive ELISA has a low specificity and cannot be used in dilute samples. However, the benefits are that there is less sample purification needed, it can measure a large range of antigens in a given sample, it can be used for small antigens, and it has low variability (167).

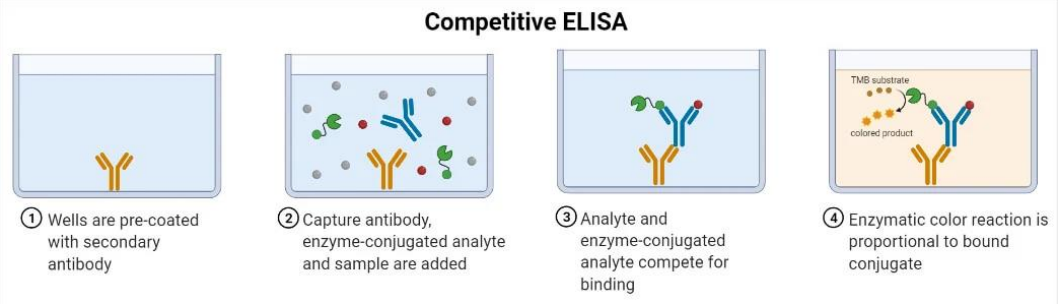



**Figure 16**: Competitive ELISA procedure.

(Adapted from: (167))

## 6.4 Influence of anxiety in medical imaging

Extreme levels of anxiety cause a high social and functional impact, with important implications for quality of life (168). Anxiety related to medical imaging exams is a well-recognized phenomenon.

Despite technological advancements, medical imaging procedures often induce patients` high levels of anxiety due to several factors (13,14,25,169):

- The possibility of diagnosing a life-threatening illness
- First-time patients' examination
- Body position during the image acquisition
- Patients' concerns with the examination result, diagnosis, and potential changes in treatment plans
- Claustrophobia
- Lack of understanding about the procedure
- Difficulties in communicating or comprehending during the examination
- The overall length of the procedure
- The use of ionizing radiation and patients' concerns about radiation exposure.
- Previous negative experiences
- High intensity of the acoustic noise

With patients experiencing anxiety during these types of medical procedures, patients' voluntary and involuntary movements increase and in turn, cause a negative impact on image quality, more specifically, the difficulty in remaining still increases the likelihood of artefact appearance, leading to misinterpretation of the image and/or the need to repeat the exam. This situation will extend the patient's stay in the medical department and alter the medical service's normal flow (70). Given the cost and growing number of medical imaging procedures, preventing anxiety and its adverse effects is essential for patients and helps conserve staff time and resources (124,170–172).

# 7. Musical Intervention

Several non-pharmacological strategies have been introduced before and during imaging procedures to minimise patient anxiety, such as meditation, massage, aromatherapy, hypnosis, and the use of music (17–20).

Music therapy is a type of therapy in which the well-being patient's development as the bond that forms between the patients and the therapist can grow quickly through musical interactions in NM environment. The definition of music intervention is unclear (173–176), although it can be divided into music therapy and music medicine. The first one referred to the psychotherapeutic use of music and required the presence of a trained therapist providing diverse personalised music experiences, such as listening to live, songwriting, and composing music (177). In music medicine, patients passively listen to pre-recorded music recommended by health professionals to address physiological or psychological needs (172). Also, (178) adheres to the following definition: "Music therapy is the professional use of music and its elements as an intervention in medical, educational, and everyday environments with individuals, groups, families, or communities who seek to optimize their quality of life and improve their physical, social, communicative, emotional, intellectual, and spiritual health and wellbeing. Research, practice, education, and clinical training in music therapy are based on professional standards according to cultural, social, and political contexts". (178)

Musical intervention is a reliable, painless, low-cost and without side effects non-pharmacological strategy (21,22). It can help maximize efforts to promote the patient's comfort and has been highly researched in numerous treatment areas of the hospital setting to study the effects on socio-emotional health (179,180). The technician uses musical improvisation, listening to music, composition of music and any other type of musical interaction to support and achieve positive changes in the patient's mood, psychological state, perceived pain and social interaction with others (172,179,181).

There are many approaches and sound repertories that technicians used during these medical procedures. Normally the researches use specific music genre such as jazz, pop, classical, etc., (131,132,136,182,183), sounds of nature (26,135,137,184,185) that are effective in patient's relaxation.

Additionally, the technical characteristics in musical intervention such as the music volume, time length, musical frequency, sound equipment and media, can be changed and adjusted to ensure better effectiveness of the intervention that the patient is exposed to (26,131–137,182–186).

To date, several systematic reviews have been conducted concerning the effect of music on diverse clinical settings and populations. Remarkably, there are systematic reviews analysing the effect of music on pain and anxiety in medical procedures (175), in surgery (187) , in burn patients during treatment procedures (188) and in children undergoing invasive surgery (180) or medical procedures (189).

Others systematic reviews focused on the impact of music on anxiety during pregnancy (190) on anxiety in general, discomfort, pain, heart rate and blood pressure in patients undergoing percutaneous coronary procedures (191), on postoperative recovery in adults (192), on psychological and physical outcomes in people with cancer (176) and on anxiety, depression, and quality of life of cancer patients undergoing chemotherapy (193).

Since patients' attention on aspects such as procedure time or symptoms can be diverted as they focus on the music (23), musical intervention can serve as a cognitive distraction technique, redirecting individuals' attention away from anxious thoughts and promoting a sense of calm (22,194).

Furthermore, several musical interventions in clinical services have induced reductions in heart rate, blood pressure (22,176,195), respiratory rate (176,195,196), pain (187,195,197) anxiety (22,187,195–199) , stress hormone levels (195) and increase patient satisfaction (200,201) and in time spent in the recommended heart rate intensity in patient stroke survivors with low gait functioning (202).

Researchers had shown that listening to music with slow-tempo and low-pitched music can induce a relaxation response and decrease anxiety levels since the rhythm and tempo of the music gradually slow down, synchronizing with the individual's breathing and heart rate (23,203–207).

Listening to preferred or personally meaningful music can divert attention and provide a positive focus, reducing anxiety levels (21,197,208). Many studies investigated the effects of patients-selected music on anxiety levels and found significant reductions in anxiety among individuals with high trait anxiety (170,194,209–211). Other researchers also showed that when patients are allowed to choose the music they listen to during medical procedures, the effectiveness of the intervention enhances observed the viability of music interventions (170).

This highlights the need for these interventions in medical imaging services, since the patient, feeling more relaxed and less anxious, reduces voluntary and involuntary movements during image acquisition and, in turn, prevent the risk of artifacts in the images and its repetition. (26,70,212–214).

# 8. Methods

## 8.1 Study Protocol

By clinical indication, we performed an MPS, 1-day protocol, rest (R)-stress(S), in the NM department and $^{99m}$TcTetrofosmin was used as a radiopharmaceutical (Figure 17).

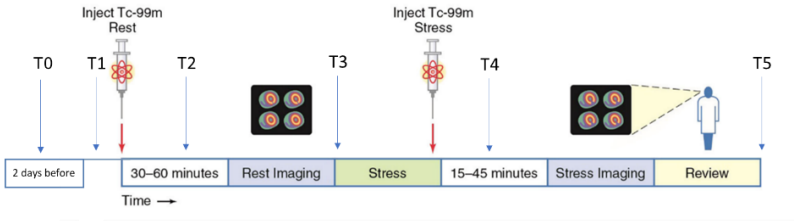



**Figure 17**: Steps of 1-day protocol for MPS exams.

The sample of patients was assessed in sociodemographic, psychological, physiological, and biochemical measures. The measurements were applied at 6 different times in MPS exam (Figure 17):

- T0: two days before the examination
- T1: when patients arrive at the NM department
- T2: before the rest image acquisition
- T3: after the rest image acquisition
- T4: before stress image acquisition
- T5: after stress image acquisition

Two studies were carried out in the project with distinct main objectives:

- Cross-sectional study: Analysis of the influence of anxiety on image quality.
- Pilot Study: Analysis of the influence of a musical intervention on participants' anxiety.

This study had to be carried out in three different NM clinic services:

- HPA- *Hospital Particular de Almada* | *NuclearMed,* **NM service 1**
- *Joaquim Chaves Saúde - Clínica de Miraflores* | **NM service 2**
- HSM – *Hospital Santa Maria* | **NM service 3**

Data collection and exams from participants corresponding to the 2 studies were carried out at:

- Cross-sectional study: NM service 1
- Pilot study: NM service 2 and NM service 3

## 8.2 Sample

### 8.2.1 Sample information

The project was carried out with a total of n=97 participants, within each study:

- CSS (n=63);
- PS (n=34): NM service 2 (n=23), NM service 3 (n=11)

When recruiting participants for the study there are several steps to follow and information to convey to participants. Adult patients, who perform 1-day protocol, rest-stress MPS at NM service was invited to participate in the study. After giving their informed consent (APPENDIX *1*), all patients who accept participation were included in the study.

To record data from the included sample, a database was created in Excel for the insertion of patient contacts, with the respective associated codes to be placed in the questionnaires and recorded data, to guarantee their anonymity.

### 8.2.2 Participants Recruitment

The type of patient recruitment depended on the type of study being carried out:

i. Cross-Sectional study:

To recruit patients for the study, it is necessary to contact them before they arrive at the hospital. In the pre-examination phase, there are 2 moments of communication with the patient:

- 1st contact: Introduction to the study;
- 2nd contact: 4 working days after sending the letter;

1st contact: Introduction to the study

We contacted the participant to explain the detailed process of the study in question. As such, the following information was given to the patient:

I. Personal presentation:
- Name;
- Professional regime;
- Study institution;
- Make the patient aware that their information was taken from the hospital database due to clinical indication, to carry out this study in myocardial perfusion scintigraphy exams.

II. Detailed presentation of the study following the information:

- Main objective: Perform myocardial perfusion scintigraphy examination with the main objective of understanding how patients' anxiety can influence the results;
- Explain the exam: Myocardial perfusion scintigraphy is an exam to diagnose heart disease and prevent its development and will be carried out in two phases: study of the myocardium at rest and study of the myocardium under stress;
- Explain arrival at the hospital:
  - Referring the patient to a room;
  - Filling out forms during the exam, in different moments;
  - Blood pressure, heart rate and oximeter measurements;
  - Salivary Cortisol tests;
  - Radiopharmaceutical administration: substance that does not harm and helps to obtain images of the myocardium. Explain it will take an average of 20 minutes for the image acquisition;
  - In the stress phase, the procedure will be the same, however, before acquiring the images, the patient will perform a stress test;
  - Explain that the patient have to remain around 6 hours in the NM department.

III. Inform you of the future calls that participants will receive to help with the pre-exam process and adjust availability with the participant.

2nd contact: 4 days after sending the letter

We contact the participant to:

I. Confirm reception of the letter with the 2 forms (Socio-Demographic and Cardiac Anxiety), the consent and the salivette;
II. Remember the participant to fill out the forms and read the informed consent;
III. Remind you about the saliva test and give the necessary instructions:

- Do not eat anything 1 hour before the test, do not brush the teeth 15 minutes before taking the exam, gargle your mouth with water;
- To carry out the test, open the blue lid of the salivette and after washing the hands, place the sponge in the tube inside the mouth. Wait 2 to 3 minutes without biting or chewing the sponge;
- After carrying out the test, store it in the refrigerator and take it to the hospital on the day of the exam.

IV. Remember the exam date and remind the patient to take with him:

- Forms completed, informed consent and saliva test performed;
- Health system card, medications, previous heart tests, request for the test with clinical information.

V. Remember that the patient:

- Can´t eat, drink or smoke 4 hours before the exam;
- 24 hours before without food or drinks containing coffee, chocolate, tea, decaffeinated drinks, Coca-Cola, ice-tea, and other similar drinks.

ii. Pilot Study:

Due to service constraints in this study, patient recruitment was only possible after their entry into the NM service. At T2, the study was explained, the patient accepted to participate and subsequently the analysis of parameters began.

### 8.2.3 Inclusion and Exclusion criteria

For this study we use different criteria to define the type of sample of participants we intended to have.

Inclusion Criteria:

- Patients with ≥18 years older;
- Patients with clinical indication for MPS in the NM department from October 2022 to June 2023;
- Patients that will perform a 1-day protocol, rest (R)-stress (S).

Exclusion criteria:

- Difficulty or inability to communicate;
- Visual or audio-visual difficulties;
- Being a foreigner and not speaking, reading, or writing the Portuguese language;
- Patients undergoing 2-day protocol or 1-day protocol – starting with stress study;
- Patients without access to internet or internet navigation technologies.

## 8.3 Measurement parameters

The sample of patients was assessed in sociodemographic, psychological, physiological, and biochemical measures. For each study, some measurement parameters were carried out at different moments during the examination:

- Cross-sectional study:
  - Socio-demographics parameters: T0 or T1;
  - Psychological parameters: STAI-S: T1 and T5; VAS: T1,T2,T3,T4 and T5
  - Biochemical parameters: T0, T1, T2 and T3
  - Physiological parameters: T1, T2, T3, T4 and T5
- Pilot study:
  - Socio-demographics parameters: T2;
  - Psychological parameters: STAI-S: T2 and T5; VAS: T2,T3,T4 and T5
  - Biochemical parameters: T2 and T3
  - Physiological parameters: T2, T3, T4 and T5

### 8.3.1 Socio-demographic parameters

Each patient was given a form with sociodemographic parameters. It contained questions about age, gender, educational qualifications, occupation, and previous medical imaging exams. The form filled out by the participants are present in APPENDIX *2*.

For a more detailed analysis of the patient's socio-demographic parameters, each patient's medical reports were accessed to consult clinical information about the number of CVRFs that patient had and their types, the amount of medication each patient take and information about the medication therapeutic group.

After collecting the participants' socio-demographic data, these were statistically analysed.

### 8.3.2 Psychological parameters

For analysed psychological measures we use in this study two different measuring instruments: State-Trait Anxiety Inventory form (STAI) and Visual analogue scale (VAS).

STAI-S

For this specific study we use the STAI-S, only with 20 items for assessing state anxiety.

All items were rated on a 4-point scale (from “Almost Never” to “Almost Always”) and the final score was evaluated on a scale of 20-80. The questions 1,2,5,8,10,11,15,16,19,20 were inverted to obtain a correct score value for each participant so that their data can later be treated statistically). The form filled out by the participants are present in APPENDIX *3*.

During the examination, for the Cross-sectional study sample, we used this measuring instrument at moments T1 and T5. Due to changes and impossibilities in services, for the sample of participants in the Pilot study we used this measurement at moments T2 and T5.

VAS

In this specific study we use the VAS to analyse the anxiety of the patients during the procedure. There are different ways of using VAS, among them are numerical rating scales, curvilinear analogy scales, meter-shaped scales and "Box-scales". For this study we use a VAS numerical scale, with 1 item rating from 0 to 10. The form filled out by the participants are present in APPENDIX *4*.

During the examination, for the Cross-sectional study sample, we used this measuring instrument from moment T1 to moment T5. Due to changes and impossibilities in services, for the sample of participants in the Pilot study we used this measurement from the moment T2 to moment T5.

### 8.3.3 Biochemical parameters

Data analysis and cortisol measurement were carried out at ESTeSL. In this study we use as a biochemical marker to anxiety in clinical practice we use a Salivary Cortisol Test. There are several brands of salivettes available to analyse cortisol. In this study we used salivettes from the SARSTEDT laboratory. Figure 18 shows the characteristics of the salivettes used.


**Figure 18:** Salivette structure and Information. The salivette contains, a blue lid, a paper label: white/blue and a roll of synthetic fibers for determining cortisol from saliva.

(Adapted from: (164))

The cortisol test for the Cross-sectional study sample was collected at T0, T1, T2 and T3, and so, each participant performed a total of 4 saliva tests during the exam. For the Pilot study, the sample was carried out at T2 and T3, and therefore, each participant performed 2 saliva tests during the exam. In both samples patients place the sponge found inside the salivette in their mouth for around 3 minutes without chewing or swallowing and then it is placed back in the tube (Figure 19). The tube was subsequently centrifuged and stored at -20ºC until dosing.

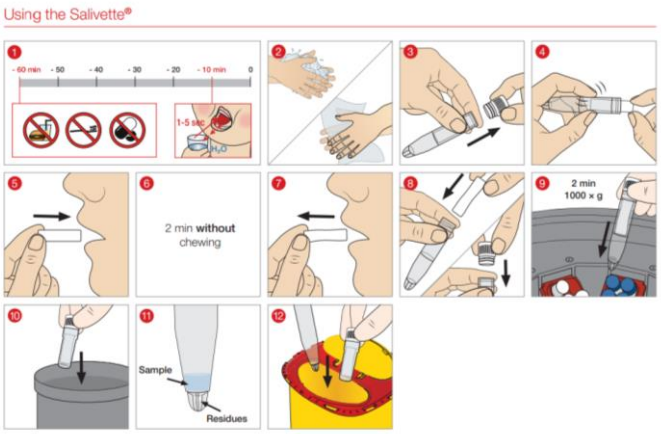



**Figure 19**: Hygienic collection of saliva samples for diagnosis and therapeutic control.

(Adapted from: (164))

Cortisol analysis was subsequently executed in a laboratory at the *Escola Superior de Tecnologia da Saúde de Lisboa*. A variety of ELISA kit formats are available. For this study we used the Cortisol Competitive Human ELISA Kit (Figure 20). Cortisol measurement was carried out on three different days, since there were many samples, and it was necessary to use 3 Cortisol Kits.


**Figure 20**: Cortisol Competitive ELISA Kit.

(Adapted from:(164))

This assay is designed to detect and quantify the level of cortisol in serum, plasma (EDTA and heparin), dried fecal extracts, urine, saliva, or tissue culture medium. Total cortisol is measured in extracted samples, serum, and plasma; while free cortisol is measured in urine, and saliva. The assay was validated with samples from human but is expected to measure cortisol in samples from other species.

The Cortisol Kit includes a 96-well plate and other components described in Table 7.

**Table 7**: Contents and storage from ELISA Kit

(Adapted from: (215)

| Components | Quantity |
|---|---|
| Cortisol Standard; 32,000 pg/mL cortisol in a special stabilizing solution | 125 µL |
| Assay Buffer Concentrate (5X) | 28 mL |
| Clear 96-well Plate, 96-well strip-well plate; goat anti-mouse IgG coated | 1 plate |
| Cortisol Antibody | 3 mL |
| Cortisol Conjugate | 3 mL |
| Dissociation Reagent | 1 mL |
| Wash Buffer Concentrate (20X) | 30 mL |
| TMB (Tetramethylbenzidine) Substrate | 11 mL |
| Stop Solution; contains 1 M HCl, **CAUSTIC** | 5 mL |
| Plate Sealer | 1 |

This Kit can be used to carry out different protocols, depending on what we want to isolate. We used the protocol for the saliva sample type, following the next steps (164):

- Prepare 1x Wash Buffer:
    1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer;
    2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.
- Prepare 1X Assay Buffer:
    1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer;
    2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.
- Prepare Samples: Dilute samples ≥1:4 with 1X Assay Buffer
- Dilute standards:
    1. Add 50 µL Cortisol Standard to one tube containing 450 µL 1X Assay Buffer and label as 3,200 pg/mL cortisol;
    2. Add 250 µL 1X Assay Buffer to each of 7 tubes labelled as follows: 1,600; 800; 400; 200; 100; 50; and 0 pg/mL cortisol;
    3. Make serial dilutions of the standard and mix thoroughly between steps.

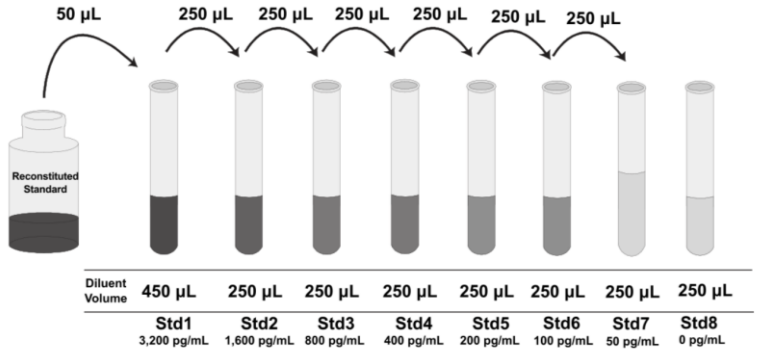



**Figure 21:** Diagram of the dilutions of the standards.

(Adapted from: (164))

- Bind antigen:
    1. Add 50 µL of standards or samples to the appropriate wells;
    2. Add 75 µL 1X Assay Buffer into wells for detecting non-specific binding (NSB);
    3. Add 25 µL of Cortisol Conjugate to each well;
    4. Add 25 µL of Cortisol Antibody to each well except NSB wells;
    5. Tap the side of the plate to mix. Cover the plate with plate sealer and incubate for 1 hour at room temperature with shaking;
    6. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.
- Add chromogen:
    1. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue;
    2. Incubate for 30 minutes at room temperature.
- Add stop solution: Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow;
- Read the absorbance at 450 nm within 10 minutes after adding the Stop Solution.

With the values from the absorbance, we generate the standard curve and calculate cortisol concentrations. As three cortisol kits were used, it was necessary to create three distinct standard curves to calculate cortisol concentrations.

For each calibration curve, standard reading values were observed and adjusted according to the range of concentrations close to the expected concentration in the sample of interest.

To create the calibration curve, an f (x, y) graphic was used. The x values represent 1/Absorbances of each reading of the standards and y values the concentrations obtained from the dilution of the standards (Figure 21).

With the graph, it is possible to plot the standard curve and obtain its 2nd degree equation, from which the concentration can be calculated (Equation (4)) and the coefficient of determination, or $R^2$, that provides information about the statistical measure and of how well the regression line approximates from the actual data.

$$y = ax^2 - bx + c \tag{3}$$

In the samples of interest, the absorbance was transformed into 1/Absorbance. With these new values, it was possible to replace x in the 2nd degree equation and obtain y values. Since the sample was diluted 1:4 during the procedure, the y values are multiplied by 4 to obtain the intended concentration values.

In literature, the most common units for cortisol concentrations are nmol/L (24,154,156,157,163). Therefore, since the concentrations we obtained through the graph were in pg/mL and the cortisol molar mass is 362.46 g/mol (216), it was also necessary to convert the concentration units using the following equations:

$$Cortisol\ Concentration\ \left(\frac{g}{L}\right) = Cortisol\ Concentration\ (pg/mL) \times 10^{-9} \tag{4}$$

$$Cortisol\ Concentration\ (mol/L) = \frac{Cortisol\ Concentration\ (g/L)}{362{,}46} \tag{5}$$

$$Cortisol\ Concentration\ (nmol/L) = Cortisol\ Concentration\ (mol/L) \times 10^{9} \tag{6}$$

As explained in before, cortisol levels during the day drops significantly and, in the evening, only about 10% of the morning cortisol remains in the body. Moreover, salivary cortisol differs with age and sex (24). We use Table 6 as a literature reference to cortisol concentrations.

The cortisol concentrations obtained were compared with each other, at different moments of the exam (T0, T1, T2 and T3), to understand their changes during the exam.

### 8.3.4 Physiological parameters

In addition to psychological and biochemical parameters, data on the patient's physiological parameters were collected. 4 different physiological parameters were used:

- Blood pressure (Sbp e Dpb mmHg)
- Heart rate (beats per min)
- Respiratory rate (breaths per minute)
- Blood Oxygen Levels ($SpO_2$%)

#### i. Blood pressure (Sbp e Dpb mmHg)

The measurements were acquired using the BBPM 4541W and HEM 742INT equipment, existing in the three services where the study was carried out. The main characteristics of the equipment are presented in Table 8. During the study, to Cross-sectional study the measurements were taken from the moment T1 to T5. To Pilot study sample, the measurements were taken from T2 to T5.

**Table 8**: Blood pressure equipments specifications

| Equipment specifications | BBPM 4541W | HEM 742INT |
|---|---|---|
| **Typology** | Wrist Blood Pressure Monitors | Arm Blood Pressure Monitors |
| **Sbp (mmHg)** | 60-260 | 299 |
| **Dbp (mmHg)** | 40-199 | 0 |
| **Arrhythmias detector** | Yes | Yes |
| **Heart rate (beats per min)** | 40-180 | 40-180 |
| **Circumference range (cm)** | 14 x 19.5 | 14.6 x 44.6 (Tube = 60) |

It is recommended that BP be classified, and hypertension graded as follows (Table 1):

- Optimal - SBP <120 mmHg and DBP <80 mmHg
- Normal - SBP 120-129 mmHg and/or DBP 80-84 mmHg
- High normal - SBP 130-139 mmHg and/or DBP 85-89 mmHg
- Grade 1 - SBP 140-159 mmHg and/or DBP 90-99 mmHg
- Grade 2 - SBP 160-179 mmHg and/or DBP 100-109 mmHg
- Grade 3 - SBP ≥180 mmHg and/or DBP ≥110 mmHg
- Isolated systemic hypertension - SBP ≥140 mmHg and DBP <90 mmHg

The participants' blood pressure data was collected and subsequently statistically analysed.

ii. Heart rate (beats per min)

During the study, to Cross-sectional study the measurements were taken from the moment T1 to T5. To Pilot study sample, the measurements were taken from T2 to T5. The measurements were acquired using the BBPM 4541W and HEM 742INT equipment, existing in the three services where the study was carried out. The main characteristics of the equipment are presented in Table 2) (47).

The participants' heart rate data was collected and subsequently statistically analysed.

iii. Respiratory rate (breaths per minute)

During the study, to Cross-sectional study the measurements were taken from the moment T1 to T5. To Pilot study sample, the measurements were taken from T2 to T5. We used reference values from the literature, which are present in the Table 3. After collecting data from participants, the data were statistically analysed.

iv. Blood Oxygen Levels ($SpO_2$%)

During the study, to Cross-sectional study the measurements were taken from the moment T1 to T5. To Pilot study sample, the measurements were taken from T2 to T5. We used reference values from the literature, which are present in the Table 4 (50). After collecting data from participants, the data were statistically analysed. The measurements were acquired using a fingertip pulse oximeter.

The participants' blood oxygen levels data was collected and subsequently statistically analysed.

## 8.4 Image acquisition characteristics

The myocardial images are obtained with the patient in the supine position, feet-first, head supported and with the arms elevated above the head, with a belt placed around the waist. (12).

Each service had different image acquisition conditions. In Table 9 it is possible to observe the acquisition characteristics used by NM service 1, where the data for the cross-sectional study were collected, and NM service 2 and 3 where the data for the pilot study were collected.

**Table 9**: Image acquisition characteristics used in the 3 NM services.

| | NM Service 1 | NM Service 2 | NM Service 3 |
|---|---|---|---|
| **Rest acquisition time (min)** | 18 | 18 | 6 |
| **Stress acquisition time (min)** | 18 | 18 | 3 |
| **Rest 99mTc activity (MBq)** | 400 | 400 | 400 |
| **Stress 99mTc activity (MBq)** | 1200 (three times more than the first injection) | | |
| **Photopeaks (keV)** | 140 | 140 | 140 |
| **Matrix (pixel)** | 64 x 64 | 64 x 64 | 64 x 64 |

The studies were acquired using GE Infinia II (GE Healthcare), Discovery NM 530c and Siemens Symbia , existing in the three NM services where data from participants were collected. The main characteristics of the three pieces of equipment included in this study are presented in Table 10.

**Table 10**: Characteristics of the 3 gamma cameras used in the study.

| Gamma Camera | GE Infinia II | Siemens Symbia | Discovery NM 530c |
|---|---|---|---|
| **Nº of detectors** | 2 | 2 | 2 |
| **Detector material** | Sodium Iodide | Sodium Iodide | CZT |
| **Detector type** | L-mode, 180º<br>45º RAO – 45º LPO | L-mode, 180º<br>45º RAO – 45º LPO | Steady |
| **Crystal thickness (mm)** | 9.5 | 9.5 or 15.9 | 5 |
| **PMTs (for detector)** | 59 | 59 | 59 |
| **FOV (cm)** | 54 x 40 | 53.3 x 38.7 | - |
| **Collimator type** | LEHR | LEHR | LEHR |
| **Collimator design** | Hexagonal | Other | Other |

## 8.5 Image Quality

For image quality analysis, only resting images from the sample of Cross-sectional study participants were analysed. To execute, we used the Xeleris 4.0 Processing and Review Workstation Software| GE Healthcare (217) and specifically for this study, the EMORY TOOLBOX tool. With AI.

To analyse the desired image quality parameters (Noise and Contrast), several steps were followed in the procedure:

- Open Xeleris 4.0 and select ECTool Box;
- Enter the patient's ID number;
- Select the Rest studies images;
- Select “slash perfusion function” to 3 projections;
- Eliminate “contour”;
- Select tool “Auto-ROI” and threshold 4;
- Plot two ROIs, one for the zone of interest and one for the background;

- Plot two ROI for each projection (short axis, long vertical axis and long horizontal axis);
- For each ROI, extract the average count and standard deviation values that are available in the program tables

To analyse the number of patients movements during the imaging acquisition, other steps were taken:

- Open Xeleris 4.0 and select ECTool Box;
- Enter the patient's ID number;
- Select the Rest studies images;
- Select “Motion correction”

The Figure 22 shows an example of the results obtained in the program, for the 3 projections of the myocardium, with the ROI of the area of interest and the background already plotted.

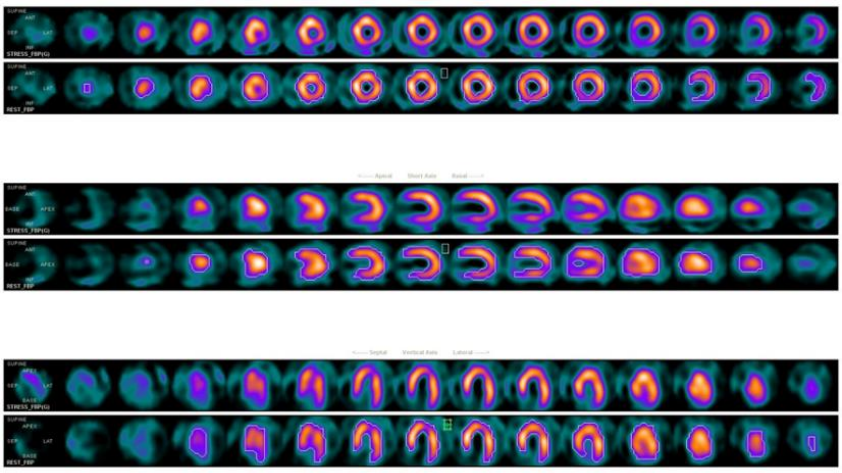


**Figure 22**: ROIs to the zone of interest and background. Three myocardial projections of one patient.

### 8.5.1 Noise

The average percentage of noise was determined for each patient's projection, using the following calculation for the data extracted from the program:

$$\%\, Noise = \frac{\sigma ROI_{Myocardium}}{NROI_{Myocardium}} \times 100 \qquad (7)$$

The $\sigma \mathrm{R}OI_{\text{Myocardium}}$ and $\bar{N}\mathrm{R}OI_{\text{Myocardium}}$ correspond, respectively, to the standard deviation and the average value of the counts in the volume of interest (Myocardium).

### 8.5.2 Contrast

The contrast of SPECT images was calculated through the following equation:

$$Contrast = \frac{NROI_{Myocardium} - NROI_{Background}}{NROI_{Background}} \quad (8)$$

The $\bar{NROI}_{\text{Myocardium}}$ correspond, respectively, the average value of the counts in the volume of interest (Myocardium) and $\bar{NROI}_{\text{Background}}$ the average value of the counts in the background.

### 8.5.3 Number of movements

The number of patient movements was obtained from the Xeleris software, with the previous procedure. In Figure 23 A), the data that the program provides after the search is represented. For each patient, with the graph represented in Figure 23 B), the number of peaks is counted (number of pixels) and for statistical analysis the movements were classified into two categories:

- Number of movements < 1 pixel: No statistically significant
- Number of movements 1-2 pixel: Statistically significant

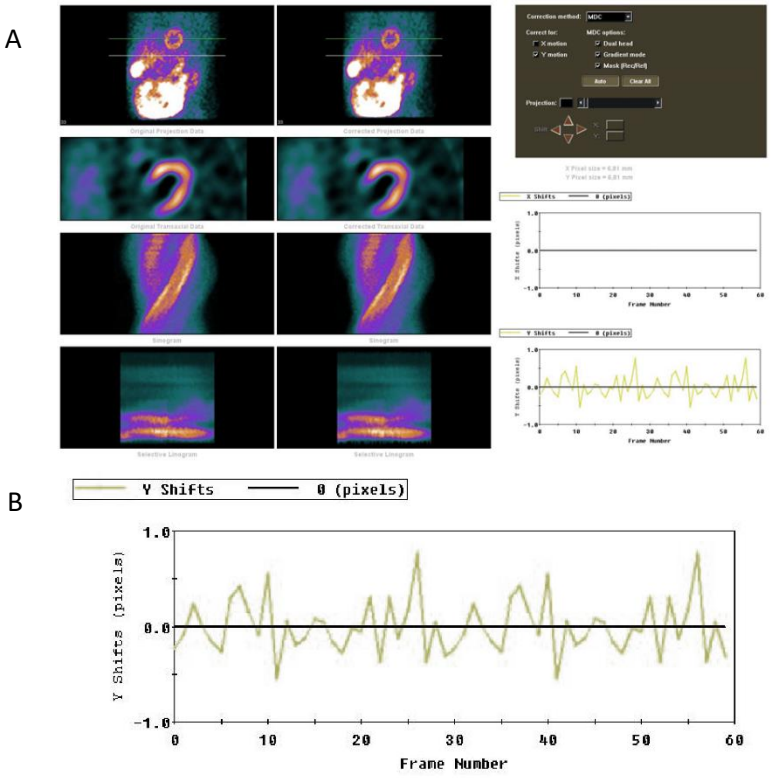



**Figure 23**: Xeleris 4.0 Processing and Review Workstation Software| GE Healthcare data. A) "Motion Correction" data of one participant; B) Number of movements in one participant.

The number of movements data for each patient during rest image acquisition was statistically analysed and correlated with other parameters.

## 8.6 Musical Intervention

The musical intervention was performed only for the Pilot study and during the rest image acquisition. The intervention time depended on the image acquisition time but was approximately 15-20 min. The computer volume was converted to dB through the application “Decibel X” (218)

To this musical Intervention, we use as a sound equipment Headphones JBL Tune 670NC Noise Cancelling Bluetooth - Blue. The general characteristics of the headphones are found in Table 11.

**Table 11**: Headphones equipment specifications

| Equipment | Headphones JBL Tune 670NC |
|---|---|
| **Frequency** | 20Hz - 20kHz \| BT: 2.4GHz – 2.4835GHz |
| **Sensitivity** | 103.5dB SPL@ 1kHz 1mW \| Microphone: -29dBV@1kHz/Pa |
| **SPL** | 98 dB |
| **Integrated Microphone** | Yes |
| **Noise-Cancelling** | Yes |

Before entering the room, the procedure was explained to patients, and they had the opportunity to make their musical choice between 5 possible options:

i. Headphones without music;
ii. Specific music theme: Debussy "La fille aux cheveux de lin"
iii. Specific music theme: Pachelbel - Canon In D Major
iv. Sounds of nature: Wind sounds
v. Personal choice

In addition to choosing music, the participant can also choose the volume of the music and, later during image acquisition, make changes if they feel discomfort.

### 8.6.1 Headphones without music

Noise suppression headphones block external noise to allow as little external noise as possible reach the patient's ears and in turn cause discomfort (219,220).

There are two types of noise cancellation used by these specific headphones:

- Active noise cancellation
- Passive noise cancellation

In active noise cancellation, there is a built-in hardware with a microphone that works as a filter. This monitors noise from the outside environment and creates an anti-noise system using sound waves created by the device itself. These waves copy the structure of the outside noise, acting as a mirror, “reflecting” this external sound wave and cancelling it (219–221).

The participant, if they didn’t want to listen to music but still wanted to reduce the noise from the outside environment, especially the noise coming from the gamma camera, could opt for this choice.

### 8.6.2 Specific music themes

i. Specific music theme: Debussy "La fille aux cheveux de lin"
ii. Specific music theme: Pachelbel - Canon In D Major

Specialist in electroacoustic music and immersive sound systems who belongs to the group of this project chose these two themes to use in this musical intervention since, according to the literature, this piece was previously associated with less stress levels, blood pressure, pulse, and body temperature (222) and classical musical is effective for reduction anxiety levels(223,224).

### 8.6.3 Sounds of nature: Wind sounds

Specialist in electroacoustic music and immersive sound systems programmed a musical track composed of different wind sounds.

### 8.6.4 Personal choice

Participants, doing a personal choice, could opt for any musical genre, sounds of nature, specific music theme, only with the condition it was relaxing music and didn’t involve too many beats to stimulate their movements. For the participant's personal choice, the Spotify Premium application was used (225).

## 8.7 Study dependent and independent variables

### 8.7.1 Independent Variables:

- GC equipment;
- Clinical indication to perform MPS Measures;
- Patient's age;
- Patient's gender;
- Patient's civil status;
- Patient's academic degree and occupation;
- Patient's previous exams;
- Physiological parameters:
    i. Heart rate
    ii. Blood pressure
    iii. Oximetry
- Biochemical parameters: Salivary cortisol levels

### 8.7.2 Dependent Variables:

- Patients Anxiety;
- Number of artifacts visualized in the image;
- Noise;
- Contrast;
- Number of patients movements;
- Musical interventions

## 8.8 Statistical Analysis

The statistical software SPSS V26.0 for Windows was used for data analysis. For sample characterization, frequency analysis (n, %) was used for qualitative data and quantitative data, central tendency measures of location (mean and median), and absolute measures of dispersion (standard deviation, interquartile range). Multilevel regression analysis was used to identify the determinants of anxiety in patients undergoing myocardial perfusion scintigraphy and its influence on image quality. Bland Altman analysis assessed the quality of the models obtained. To compare the anxiety levels across the 5 measurements, repeated measures ANOVA was used. To assess the influence of anxiety on image quality Poisson or Negative Binomial regression was used.

The study was approved by the ESTeSL Ethics Committee, with the following reference IPL/2022/CPMeAQI_ESTeSL. All patients agreed to be included in the study, through oral and written informed consent APPENDIX 1.

# 9. Results

## 9.1 Introduction

Three phases were carried out in the project, the first phase referring to the analysis of the Cross-sectional study, the second phase the analysis of the Pilot study and the third phase the comparison between these two studies.

The two studies are composed by a heterogeneous sample of participants. The sample of participants in the Cross-sectional study represents 64.9% of all participants and participants in the Pilot study group represent 35.1% (Figure 24). Table 12 shows the ages of the participants in the two studies based on data from APPENDIX 5 and APPENDIX 17.

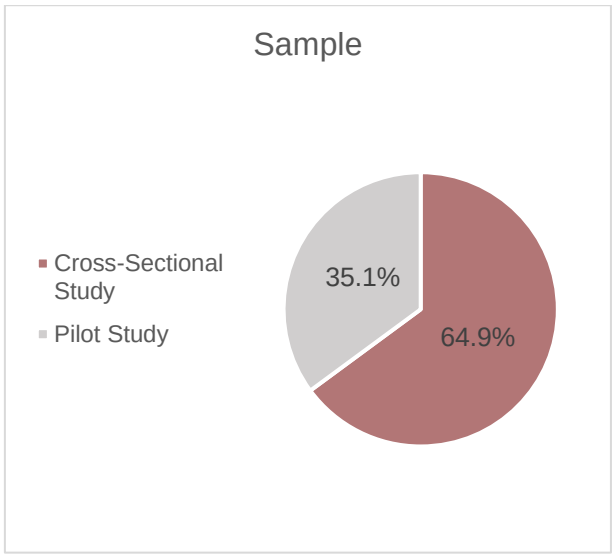



**Figure 24**: Number of participants (%) in Cross-sectional study and Pilot study.

**Table 12:** Minimum, maximum, and mean of participants age in the Cross-sectional study and Pilot study.

| Age | Minimum | Maximum | Mean |
|---|---|---|---|
| **Cross-sectional study** | 37 | 88 | 67 |
| **Pilot study** | 34 | 84 | 64 |

In this chapter the results were analysed according to the three phases of the study. In the different sections, psychological, biological, physiological, and sociodemographic measurement parameters will be analysed. Image quality analysis was performed only for the Cross-sectional study and is therefore found in section 8.1.

## 9.2 Cross-sectional study

### 9.2.1 Sociodemographic parameters

The Cross-sectional study consists of a total of 63 participants. Data was collected from participants in NM service 1. In the first phase of the Cross-sectional study, the sociodemographic data of the sample of participants was analysed. Table 13 shows the distribution of patient ages according to the gender of participants represented in Figure 25. The data were taken from the APPENDIX 5.

**Table 13:** Different ages groups with number of male and female participants

| | [30-39] | [40-49] | [50-59] | [60-69] | [70-79] | [80-89] | Total |
|---|---|---|---|---|---|---|---|
| **Male** | 0 | 2 | 5 | 9 | 13 | 6 | 35 |
| **Female** | 2 | 1 | 3 | 7 | 14 | 1 | 28 |

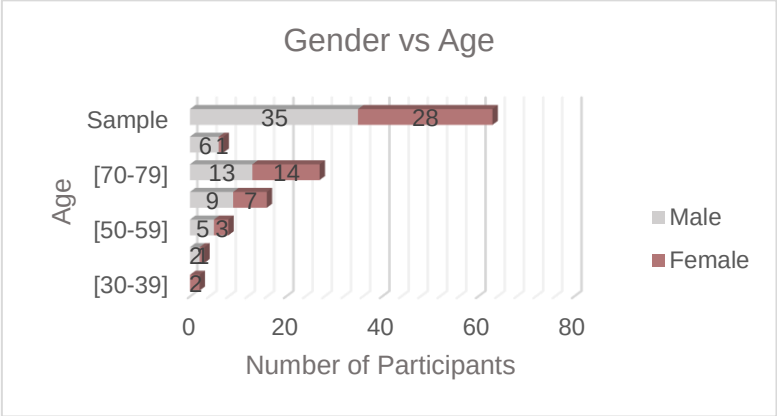



**Figure 25:** Distribution of female and male participants in different age groups.

We found that there are a total of 35 men and 28 women in the sample, the majority of whom are aged between 70 and 79 years old.

Then it was checked whether participants had already undergone other medical imaging exams prior to this one and compared them with the gender of the sample Figure 26. The data was taken from the APPENDIX 5.

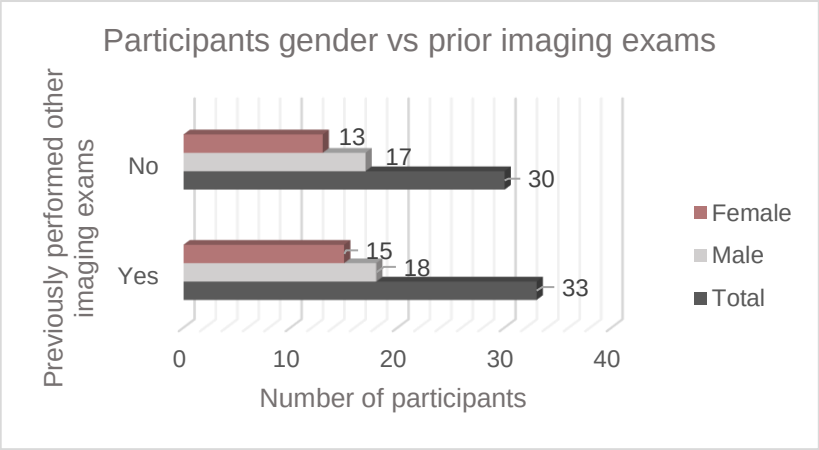



**Figure 26:** Comparation between participants gender by carry out previous imaging exams.

We found that a larger sample of participants had previously undergone exams (n=33) than those who had not yet (n=30). In both situations, the number of male participants was higher.

Of the participants who had already taken imaging exams, we checked the distribution of the degree of difficulty that each participant classified in their previous experiences, with 0 being considered as “Very difficult” and 10 as “Very easy”. The data was taken from APPENDIX 5. and are represented in Figure 27.

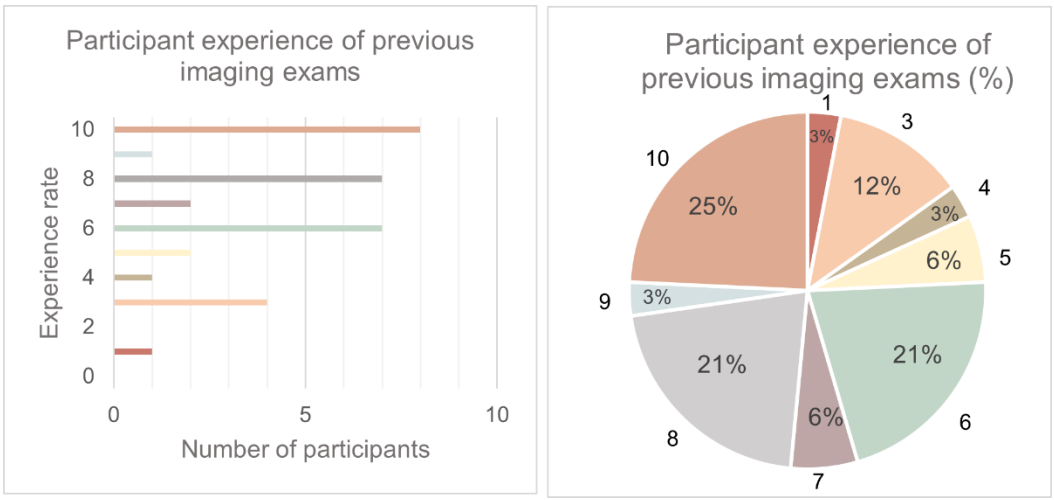



**Figure 27**: Different levels of previous exams difficulty and the number of participants.

We found that the rating option 10 “Very easy” was the most chosen by participants (25%) and most participants rated previous exams with values higher than 5, thus showing that participants had a positive experience in previous exams of medical imaging.

The study initially intended to understand whether the participant having already worked in the health sector was a significant factor in their anxiety during the exam.

As in the total sample, only one participant worked in the area, it was not considering a significant value to carry out comparative tests with anxiety factors.

The number of risk factors for cardiovascular diseases that each patient had was analysed. The number of risk factors for each patient varied from 0 to a maximum of 5 risk factors that the patient had, that is, participants could have more than one risk factor recorded in their medical report. There was also a percentage of participants without information in their medical reports about the number of CVRFs or their type. Information on the number of risk factors for each participant and their types can be found in Figure 28, based on data in APPENDIX *5*.

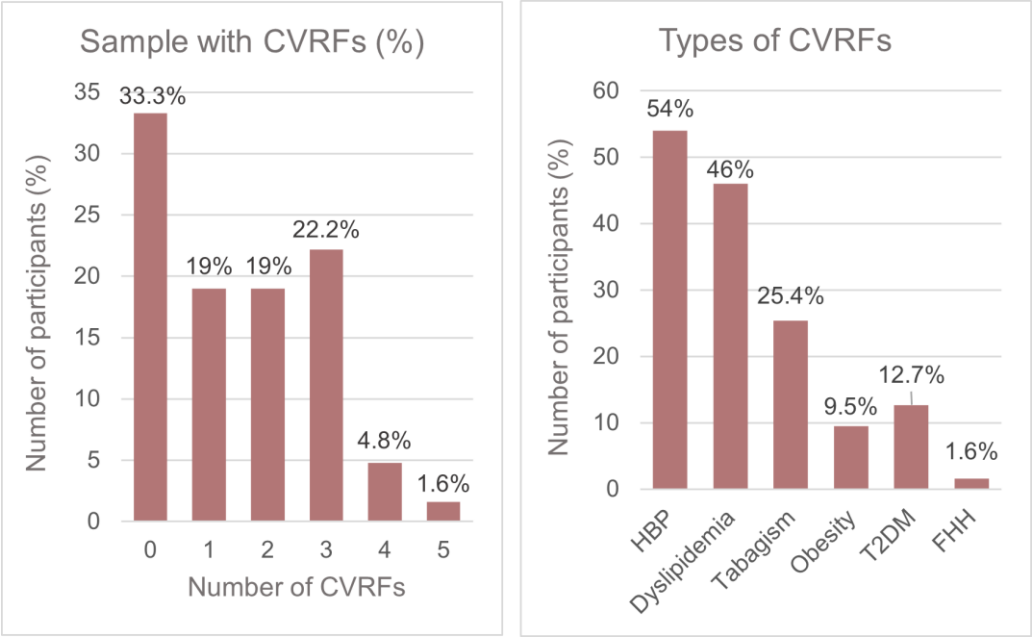



**Figure 28:** Number of CVRFs and its types compared with number of participants in each.

We observed from Figure 28 that in most participants did show any associated cardiovascular risk factors (33.3%). To the participants who had CVRFs, 22.2% had 3 cardiovascular risk factors. Furthermore, we found that for the participants with associated CVRFs, 54% had BPH and 46% Dyslipedemia. The risk factor FHH (family health history) was only presented by one participant, corresponding to 1.6% of the total sample.

The following Figure 29 refers to the patients' medication. It shows the number of medications the patients were taking and the therapeutic groups to which this medication belonged. The number of patients for each condition is presented in (%).

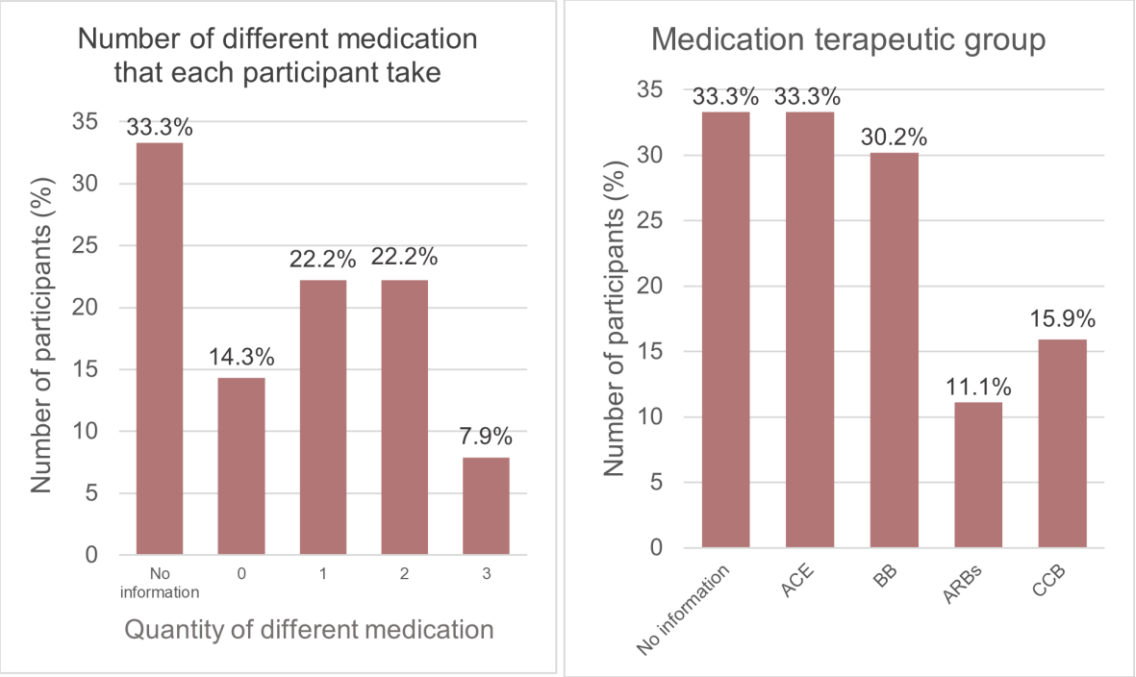



**Figure 29:** Number of different medication and its types comparing with the number of participants in each.

We see that from Figure 29 most participants (33.3%) didn't contain information about their medication in the clinical report. Of those who presented clinical information, 22.2% were taking 1 type of medication or 2 different types of medication for cardiovascular diseases. Of the participants taking medication, 33.3% needed to take ACE type medication and 30.2% needed BB type medication. Only 11.1% of participants were taking ARBs type medications and 33.3% of participants were unable to verify whether they needed medication as this information was not available in their report.

### 9.2.2 Psychological parameters: STAI

The STAI-S, as verified in the literature, is a test that is directly related to anxiety. Higher scores obtained in these exams are related to higher levels of anxiety that the patient feels in the moment. The different STAI score values of the participants across the 2 measurement moments for this sample were represented in Figure 30 based on data from APPENDIX 6.

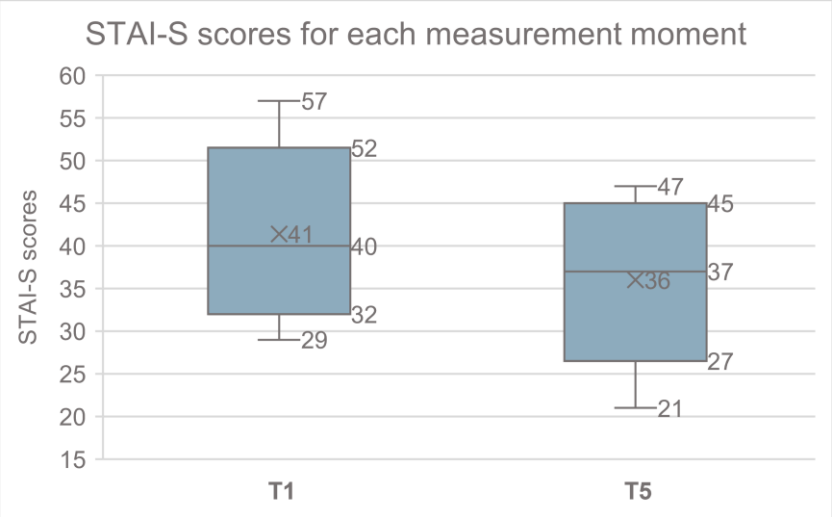



**Figure 30**: STAI-S scores do different exam moments.

In the figure above, we represent the STAI-S values per moment through a boxplot. For each moment, the minimum, average and maximum value of the participants' score was obtained and the values of Q1 (middle number between the smallest number minimum; 25% of the data is below this point) and Q3 (middle value between the median and the maximum of the data set; 75% of the data lies below this point. We found for T1 that the average score was approximately 41.3, with a minimum value of 29 and a maximum of 57. For T5 we observed an average score of 37, a maximum score of 47 and a minimum of 21. Comparing the times, we can observe that there is a decrease in scores from T1 to T5 and thus a decrease in anxiety between moments.

Comparing the average scores with the reference score classifications (State and Trait Anxiety Scores of Patients Receiving Intravitreal Injections) we found that for time T1 the average score is classified as “moderate anxiety” and for T5 as “no or low anxiety”).

As STAI scores are higher at T1 than at T5 and, considering the objective of the study to understand the influence of anxiety on patients during MPS exams, the correlation of STAI psychological data with the patients' socio-demographic parameters will be only referring to moment T1. Furthermore, we will only use average values of the scores at T1.

### 1. STAI-S and Gender

Firstly, we intend to check whether there is any relationship between the anxiety parameters and the gender of the participants. The results are shown in the following Figure 31 based on data from APPENDIX 5, APPENDIX 6 and APPENDIX 7.

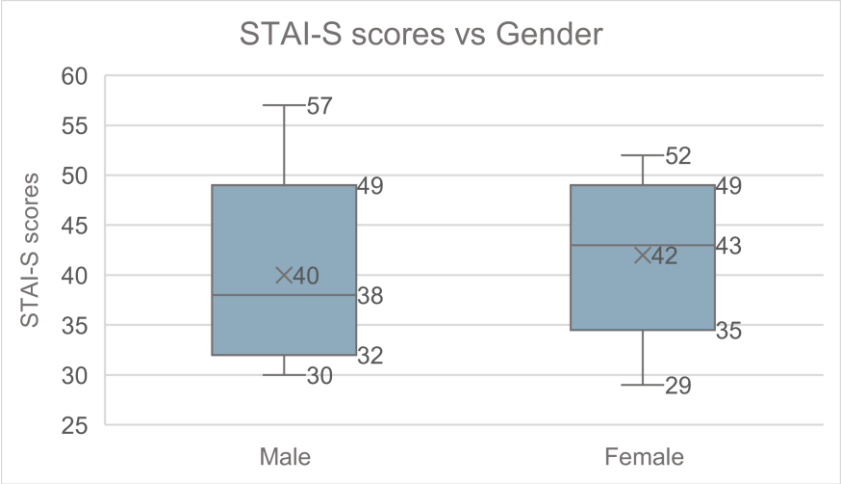



Figure 31: STAI-S scores comparing with participant's gender.

We observe that the average score for men was approximately 38, with a minimum score of 30 and a maximum of 57. For women, we observed an average score of 43, a maximum score of 52 and a minimum of 29. Comparing the times, we can see that although women had lower maximum scores than men, it was the gender that presented higher STAI-S scores in medicine and, in turn, higher anxiety values at T1.

2. STAI-S and prior imaging exams

For a more detailed analysis of anxiety in sociodemographic factors, we also considered the factor of participants having already undergone other imaging exams (Figure 32) based on data from APPENDIX 5,APPENDIX 6, APPENDIX 7.

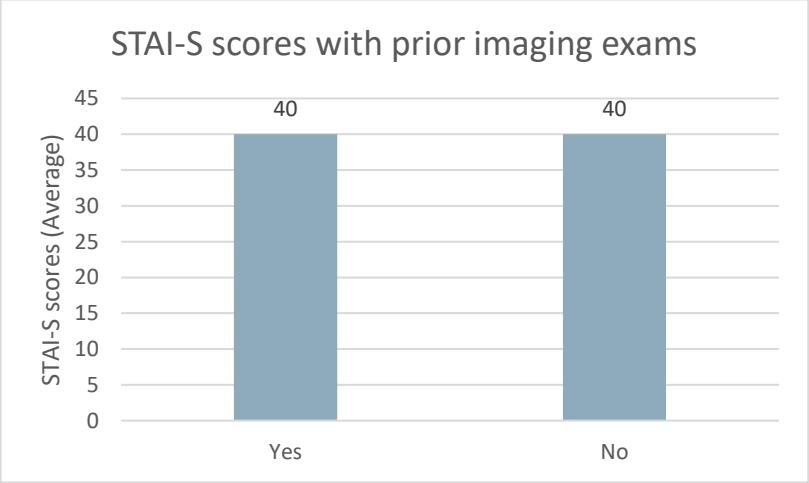



**Figure 32**: STAI-S scores compared with participants prior imaging exams.

We can see from the graph above that either the participants who had already undergone medical imaging exams or the participants who had never undergone one, presented an average score value of 40, classified as “moderate anxiety” and thus none of the groups presented anxiety values superior compared to the other.

### 3. STAI-S and academic degree

Anxiety values, as the literature proves (226) are associated with participants' lack of knowledge. In this way, we created a comparative graph between mean STAI-S values and the patients' educational qualifications (Figure 33). The results are based on data from APPENDIX 5 and APPENDIX 6.

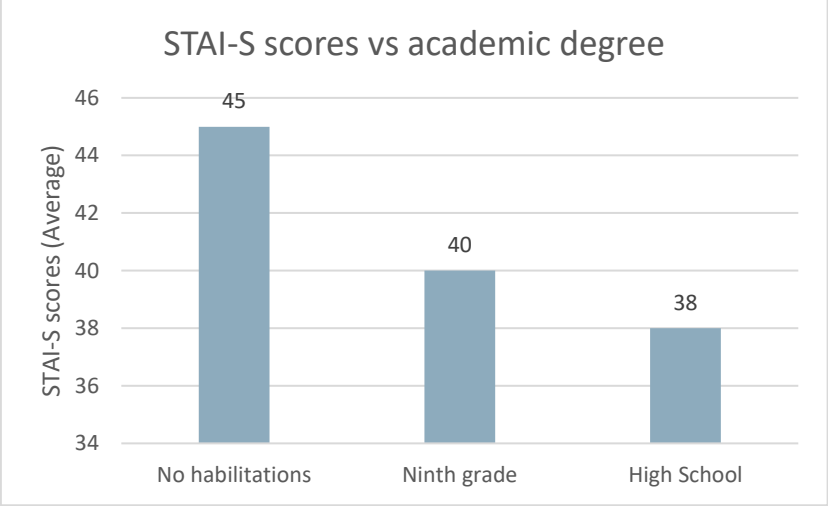



**Figure 33:** STAI-S scores with participants habilitation.

Consider the graph above, for participants without educational qualifications the average STAI-S score was 45, for participants with ninth grade the score was 40 and for participants who had secondary education the score was 38. In the sample there was only 1 participant with secondary education, and none had a bachelor's degree, master's degree or PhD, so these data were not considered in the statistical analysis. We therefore observed that the participants' score increases the lower the patients' educational qualifications, and therefore, patients with less education presented higher anxiety values.

## 4. STAI-S and CVRFs

In addition to these factors, the number of risk factors for cardiovascular diseases that each patient has can be a factor influencing anxiety. Therefore, we also draw a graph that compares these two parameters. To verify the relationship between patients' medication and the number of CVRFs, we included in the graph below the number of medications from different therapeutic groups that patients with different amounts of CVRFs have Figure 34.The results are based in data from APPENDIX 5 and APPENDIX 6.

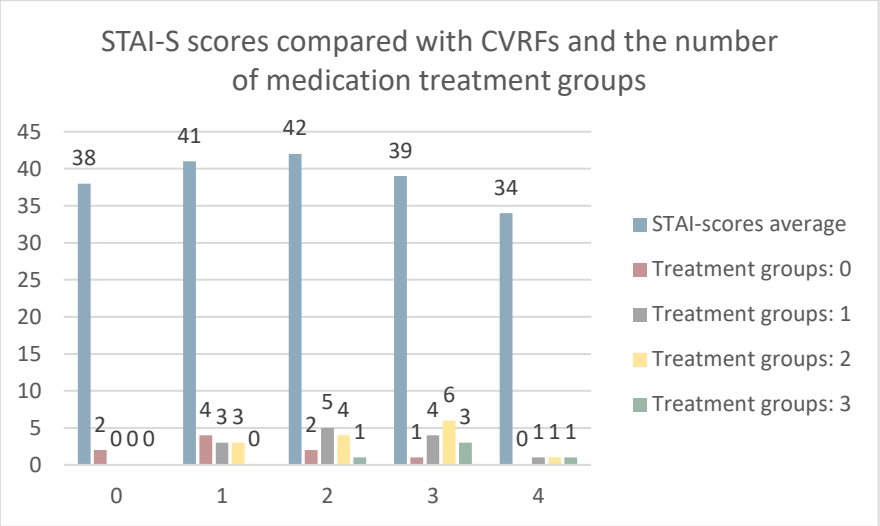



**Figure 34:** STAI-S scores compared with CVRFs and medication treatment groups.

Observing the figure above, the average score for patients with 0 risk factors is 38, for patients with 1 CRVFs it is 41, for 2 it is 42, for 3 CRVFs 39 and for patients with 4 risk factors it is 34. We found that up to 2 risk factors there is an increase in the STAI-S score and therefore an increase in patients' anxiety according to the number of risk factors associated with them. The same did not happen for patients with 3 risk factors and 4 CVRFs as they suffered a decrease in scores, that is, a decrease in their anxiety. There was only 1 participant in the sample with 5 risk factors, so these data were not considered in the statistical analysis. We also note from the graph above that in relation to the number of different medications that patients take, patients with 3 CVRFs are those with the most patients on two types of medication or 3 types of medication.

### 9.2.3 Psychological parameters: VAS

The VAS, such as STAI-S, is a test related to anxiety and the increase in scores obtained are related to higher levels of anxiety that the patient feels (150,151). All VAS results are based on data from the APPENDIX 5, APPENDIX 8 and APPENDIX 9.

The Figure 35 represents the different VAS score values of the participants over the 5 measurement moments for this sample.


**Figure 35**: VAS scores for different exam moments.

The VAS was represented in different measuring moments. We verified for T1 that the average score was approximately 5, with a minimum value of 0 and maximum of 10. For T2, an average score of 4 was obtained, a minimum value of 0 and a maximum of 10. For T3, an average value of 3, minimum value of 0 and maximum of 9. For T4 an average value of 4, minimum of 0 and maximum of 10. Finally, for T5 we observed an average score of 2, a maximum score of 9 and a minimum of 0. Comparing the time, we can observe that there is a decrease in scores from T1 to T3 and thus a decrease in anxiety between moments. We also observed, comparing T1 with T5, that there is a marked decrease in scores, that is, a decrease in patients' anxiety during the examination period. From T3 to T4 we also saw an increase in patient scores.

Comparing the average scores with the reference score classifications (151,152) we found that for time T1 the average score is classified as “Mild -Moderate”, and in the remaining times as “Mild”.

To compare VAS scores according to sociodemographic parameters, the same patient parameters were considered as in the analysis with STAI-S and, as in this analysis, the analysis of the correlation of VAS psychological data with sociodemographic parameters will only refer to the moment T1 since it was the moment where he presented higher values of anxiety in patients Figure 35.

### 1. VAS and Gender

Firstly, we intend to check whether there is any relationship between the VAS psychological parameter and the gender of the participants to understand whether the gender of the participants has an influence on their anxiety. The results are shown in the following Figure 36.

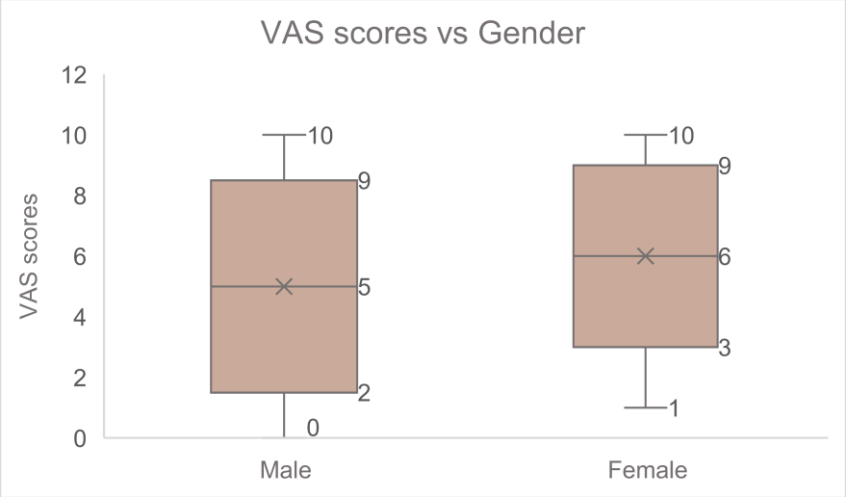



**Figure 36**: VAS scores with participant's gender.

The average score for men was approximately 5, with a minimum score of 0 and a maximum of 10. For women we observed an average score of 6, a maximum score of 10 and a minimum of 1. Comparing the times, we can see that the female gender presented, on average, higher VAS scores and, in turn, higher anxiety values at T1.

### 2. VAS and prior imaging exams

For a more detailed analysis of anxiety in sociodemographic factors, we also considered the factor of participants having already undergone other imaging exams. The graph is presented below (Figure *37*).

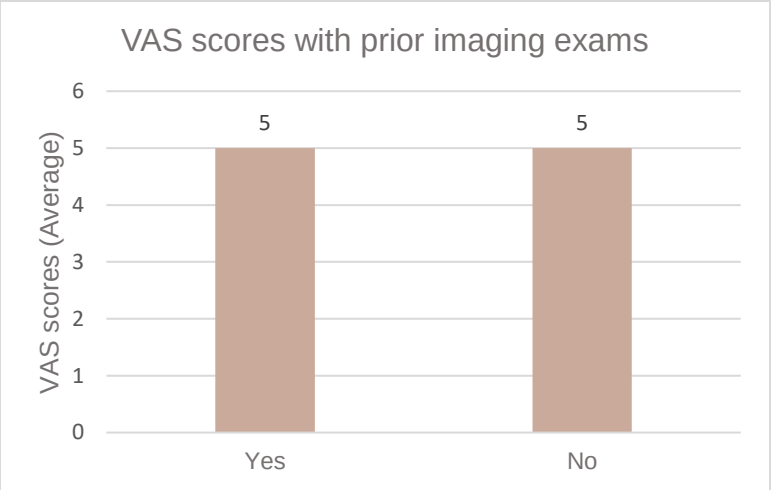



**Figure 37**: VAS scores compared with participants prior imaging exams.

We can see from the graph above that either the participants who had already undergone medical imaging exams or the participants who had never undergone one, presented an average score value of 5, classified as “Mild-moderate” and thus none of the groups presented values of higher anxiety compared to the other.

### 3. VAS and Academic degree

VAS exam anxiety values, such as in STAI-S, may also be associated with participants' lack of knowledge (226). Below is a comparative graph between average VAS values and the patients' educational qualifications (Figure *38*).

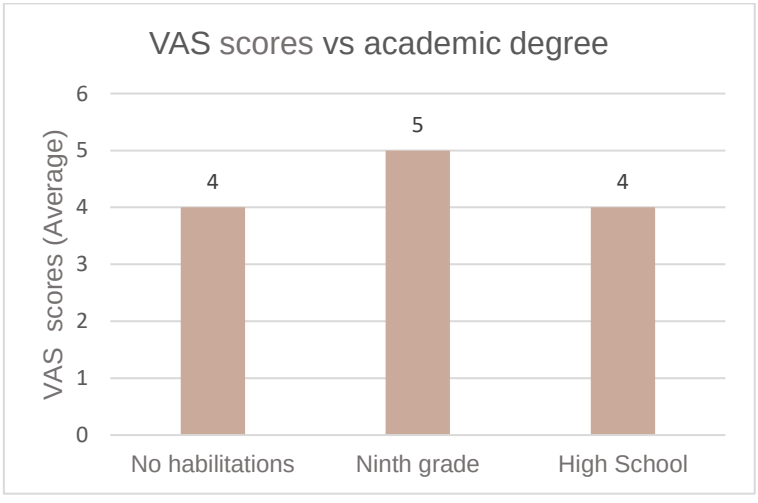



**Figure 38:** VAS scores with participants academic degree

We can see from the graph above that for participants without educational qualifications the average VAS score was 4, for participants with ninth grade the score was 5 and for participants who had secondary education the score was 4. In the sample there was only 1 participant with secondary education, and none had a bachelor's degree, master's degree, or PhD, so these data were not considered in the statistical analysis. We therefore observed that the participants' score is higher in participants with “Ninth grade” and for participants without qualifications and with secondary education they have a score of 4.

## 4. VAS and CVRFs

As the number of risk factors for cardiovascular diseases that each patient has can be a factor influencing anxiety, it was important to understand their influence by comparing with the STAI measurement but also with the VAS. Therefore, we also draw a graph that compares these two parameters Figure 39. Furthermore, we also compared the influence of the number of medications/participant ratio in participants with different amounts of CVRFs with this psychological parameter.

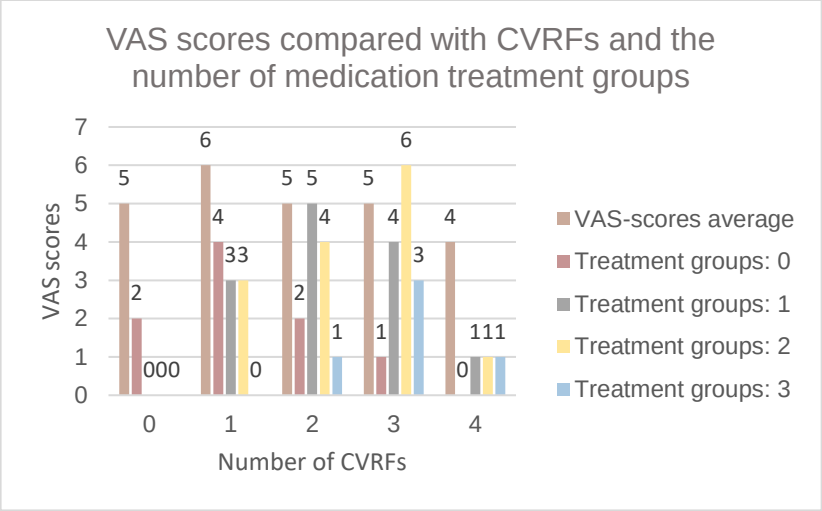



**Figure 39:** VAS scores compared with CVRFs and medication treatment groups.

The average score for patients with no risk factors is 5, for patients with 1 CVRFs it is 6, for 2 it is 5, for 3 it is 5, and for patients with 4 risk factors it is of 4. We found that up to 1 risk factor there is an increase in the VAS score and therefore an increase in patients' anxiety according to the number of risk factors associated with them. The same did not occur for patients with 2 risk factors or more, since participants with 2 and 3 risk factors did not experience changes in scores and participants with 4 risk factors had the lowest score. There was only 1 participant in the sample with 5 risk factors, so these data were not considered in the statistical analysis.

5. VAS and STAI-S correlation

To analyse the accuracy of both psychological parameters used, a comparison was made between them to check whether they have a statistically significant correlation. In this way, we use Pearson's correlation coefficient to measure the degree of correlation between the two variables. The results are in Table 14 and Table 15.

**Table 14**: Correlation between STAI-S values and VAS values, for moment T1 in the Cross-sectional study sample.

| | | **VAS_T1** |
|---|---|---|
| **STAI- S T1** | Pearson Correlation | **.341**** |
| | Sig. (2-tailed) | 0.006 |
| | N | 63 |

**Table 15**: Correlation between STAI-S values and VAS values, for moment T5 in the Cross-sectional study sample.

| | | **VAS_T5** |
|---|---|---|
| **STAI-S_T5** | Pearson Correlation | **.430**** |
| | Sig. (2-tailed) | 0.000 |
| | N | 63 |

Seeing the results, we found that for both T1 and T5 there is a statistically significant correlation between the two psychological parameters. However, it should be noted that the two values are not close to 1 (strong significant correlation between two variables), that is, in the results between the two parameters there is a statistically significant correlation but of low intensity.

### 9.2.4 Biochemical parameters

1. Cortisol with measurement moments

Cortisol, as verified in the literature, is a biomarker of anxiety (153). Since some patients didn't do the saliva test properly, the sample of participants used to measure biochemical parameters was reduced (n=47) due to the small amount of saliva we were able to collect from some participants.

The samples were measured on three different days and the concentrations were based on three different calibration curves. All biochemical parameters results are in Figure 40 and were based on data presented in APPENDIX 5,APPENDIX 10 and APPENDIX 12.

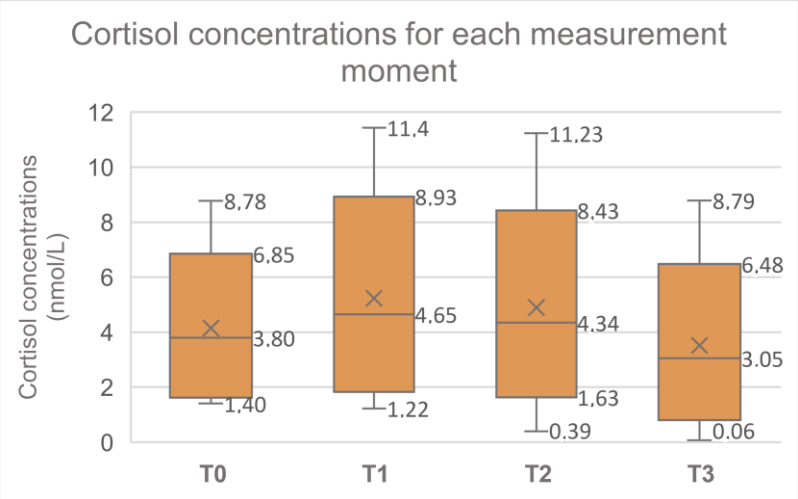



**Figure 40**: Cortisol concentrations to different moments of exam.

We represented the values of cortisol concentrations in different measurement moments. We found that for T0, carried out 2 days before the exam, the average concentration was approximately 3.80, with a minimum value of 1.40 and a maximum of 8.78. For T1, an average concentration of 4.65 was obtained, a minimum value of 1.22 and a maximum of 11.44. For T2 an average value of 4.34, minimum value of 0.39 and maximum of 11.23. For T3 an average value of 3.05, minimum of 0.06 and maximum of 8.79. Comparing the times, we can observe that there is a decrease in concentrations from T1 to T3 and thus a decrease in anxiety between moments. We also observed that time T0 compared to times T1 and T2 mostly has lower concentration values. Therefore, knowing that according to the literature, cortisol concentration values vary depending on an hourly interval, but that during 9am and 11am approximate values remain, we draw the graph that compares the average value of concentrations in T1 within this interval and outside this interval and the number of patients who had their appointment within this time compared to the number of patients who were delayed in service.

Reference values are presented for morning cortisol concentrations and afternoon/evening cortisol concentrations (24). As in our sample there are different appointment times for patients, we checked how many patients had measurement times in the morning (before 1 p.m.) and in the afternoon (after 1 p.m.) (Figure 41).

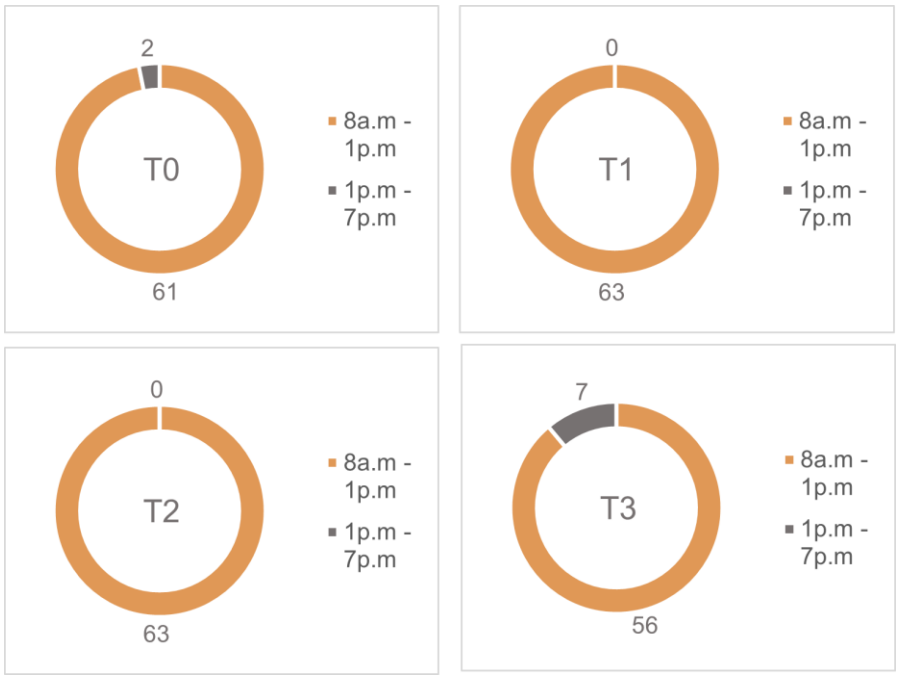



**Figure 41**: Cortisol concentrations in different moments of the exam comparing the number of participants with different schedules.

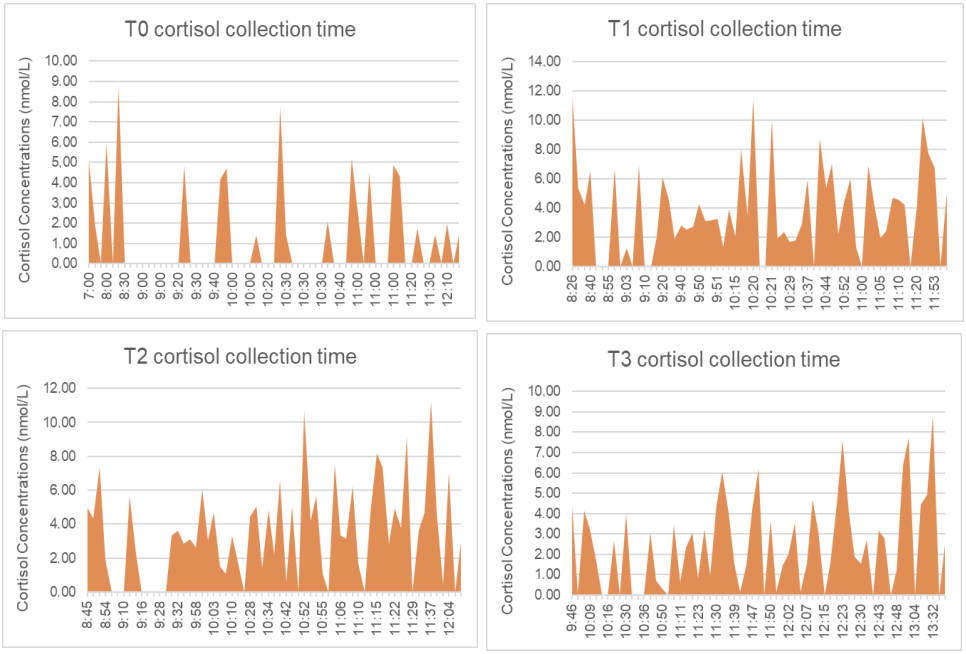



**Figure 42**: Comparison between collection time and cortisol concentrations, for each exam moment.

We observed from Figure 42 that most participants performed these 3 moments of the exam in the morning.

Also considering that there may be delays in the service throughout the exam, we then checked for the different times of the exam, whether there are significant differences in cortisol concentration Figure 42. We verified that for T0, T1 and T2 there are differences in cortisol concentrations, but all measurements were carried out in the morning. For time T3, some appointments have already been made in the afternoon. Checking the Figure 41 the number of participants who underwent exams after 1pm in T3 was only 7, a low sample, so we do not consider the concentrations of these participants to be statistically relevant.

## 2. Cortisol with Age and Gender

The correlation of cortisol biochemical data with the patients' socio-demographic parameters was only related to the T1 moment. In addition to the factors analysed above, cortisol concentrations also vary according to the participant's gender and age (24). Figure 43 and Figure 44 shows the relationship of these factors.

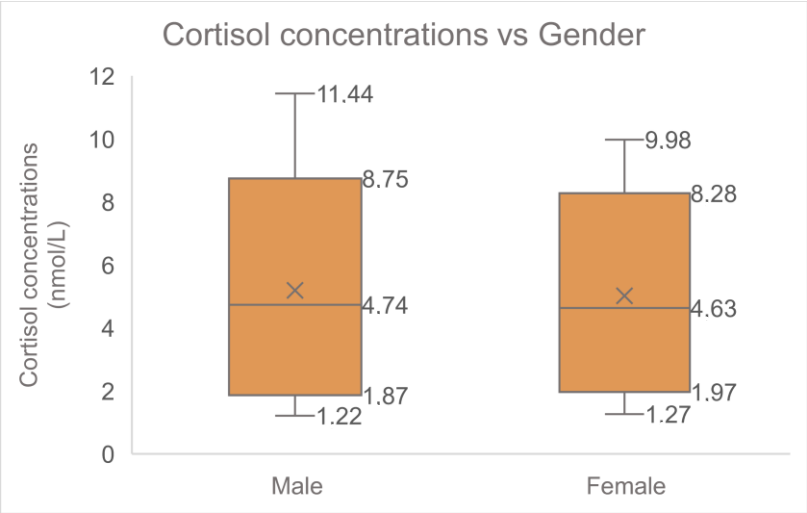



**Figure 43**: Cortisol concentrations in T1 comparing with participant's gender.

Looking at Figure 43 we found that the average cortisol concentration for men was approximately 4.74 nmol/L, with a minimum value of 1.86 nmol/L and a maximum of 11.44 nmol/L. For women, we observed an average concentration of 4.63 nmol/L, a maximum of 9.98 nmol/L and a minimum of 1.97 nmol/L. Comparing the times we can see that the male gender was the one that presented, on average, higher concentrations of cortisol, however, it is an average value very close to the value obtained for the female sample.

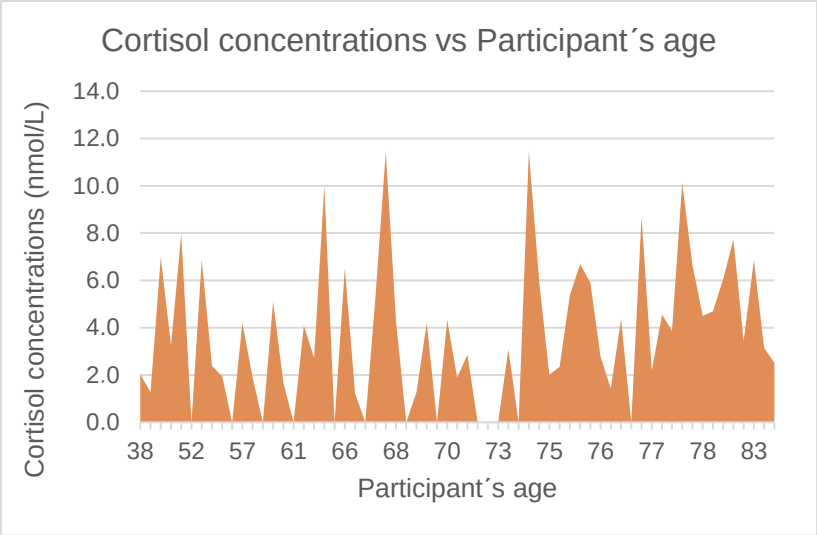



**Figure 44**: Cortisol concentrations in T1 comparing with participant's age.

According to the literature, the average cortisol concentration for people under the age of 50 is approximately 11 nmol/L and for people over the age of 50 it is 12noml/L. In Figure 44 we observe that in our sample the concentration values were lower than the values reported in the literature (24). Considering that there were low cortisol concentration values for the exam participants, we did not compare this biochemical factor with any other sociodemographic factor.

### 3. Cortisol and psychological parameters

To study biochemical parameters more accurately, this parameter was correlated with the psychological parameters, STAI-S and VAS. The data are presented in Table 16.

**Table 16:** Correlation between biochemical parameters and psychological parameters, in the Cross-sectional study sample.

| | | STAI_S_T1 | VAS_T2 |
|---|---|---|---|
| **Cortisol** | Correlation Coefficient | -0.187 | 0.019 |
| | Sig. (2-tailed) | 0.198 | 0.895 |
| | N | 49 | 49 |

Observing the results, we verified that both in the comparison of biochemical parameters with the psychological parameter STAI-S and in the comparison of biochemical parameters with the psychological parameter VAS, there was no statistically significant correlation between these meters (p=-0.187 and p=0.019, respectively).

### 9.2.5 Physiological parameters

Physiological parameters are the last parameters to be evaluated in the Cross-sectional study sample. These parameters were also measured throughout the 5 study moments. Blood pressure, heart rate, breaths/min and $SPO_2$% levels were used as physiological parameters. All physiological parameters results were based on data from APPENDIX 5, APPENDIX 13 and APPENDIX 14. With the statistical data presented in APPENDIX 14, the Figure 45 represents the different physiological parameters throughout the 5 measurement moments for this sample.

Observing the results, we verified that both in the comparison of the biochemical parameters with the STAI-S psychological parameter and in the comparison of the biochemical parameters with the VAS psychological parameter, there was no statistically significant correlation between these meters (p=-0.187 and p=0.019, respectively).

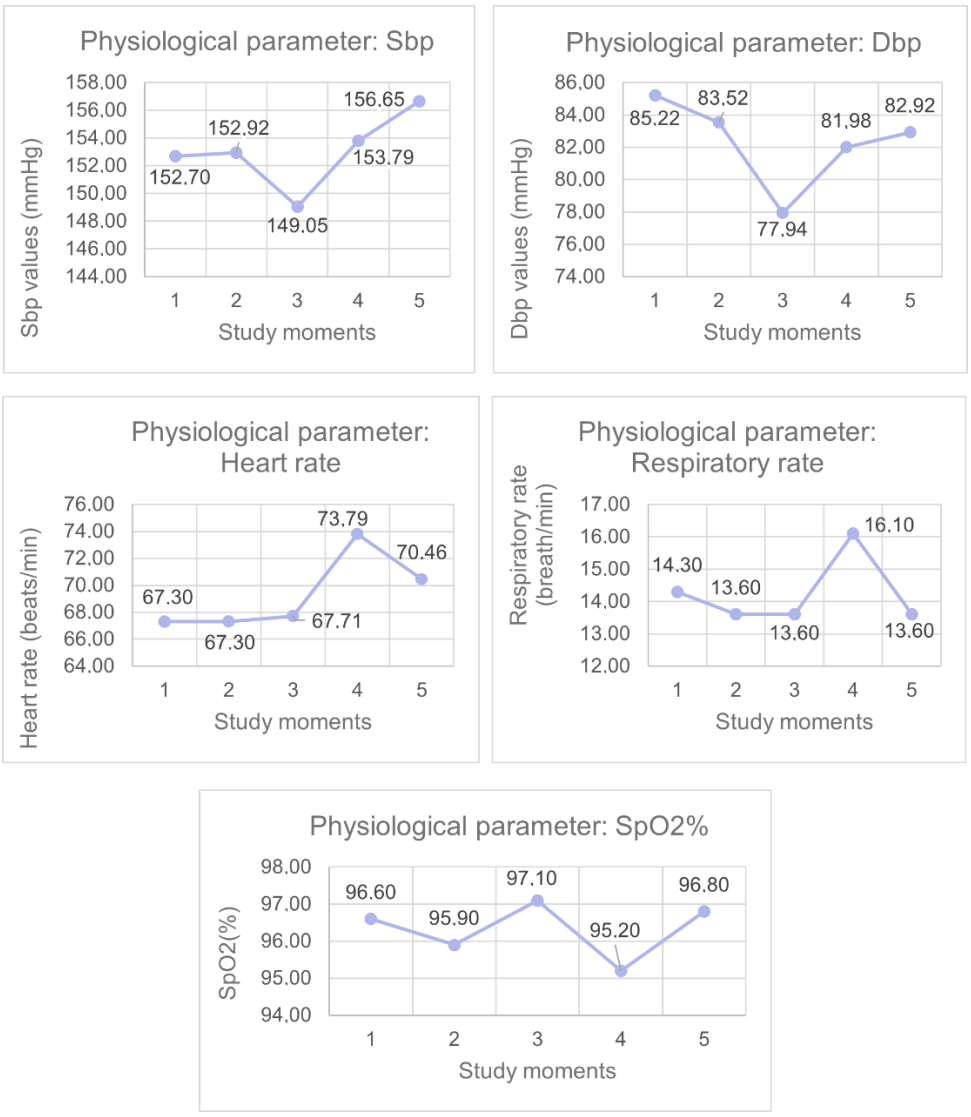



**Figure 45**: Measurements of physiological parameters for different moments of the exam.

Looking at the previous graphs, we found that the T1 time had an average blood pressure of 153/86 mmHg, an average heart rate of 67 beats/min, respiratory levels of 14 breaths/min and $SPO_2$% of 96%. For T2 a blood pressure of 153/84 mmHg, average heart rate of 67 beats/min, respiratory levels of 13 breaths/min and $SPO_2$% of 96%. For T3 a blood pressure of 149/78 mmHg, average heart rate of 67 beats/min, respiratory levels of 14 breaths/min and $SPO_2$% of 97%. We noticed a marked change in T4 values where medical participants had a blood pressure of 154/82 mmHg, average heart rate of 73 beats/min, respiratory levels of 16 breaths/min and $SPO_2$% of 95%. Finally, at T5, a blood pressure of 157/83 mmHg was observed, an average heart rate of 70 beats/min, respiratory levels of 14 breaths/min and $SPO_2$% of 97%.

As some physiological parameters are influenced by patients' sociodemographic factors, we checked the relationship between heart rate and patient age and blood pressure with the number of risk factors associated with patients. To this end, only the values obtained at T1 were considered. The data are represented in Figure 46 and Figure 47.

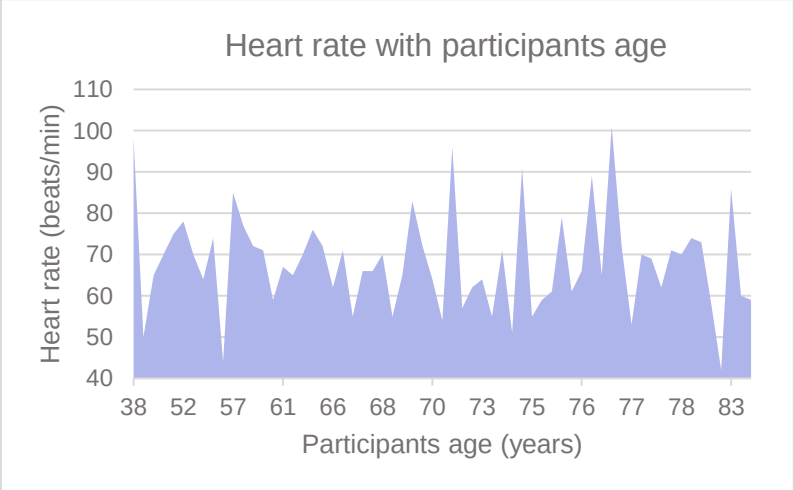



**Figure 46**: Hearts rate values comparing with participant´s age.

We verified from the results above that the heart rate undergoes some changes throughout the sample, with its average being 68.05 beats/min, a minimum of 42 beats/min and a maximum of 101 beats/min. Comparing with the literature values present in the (47), we observed that in the sample, for different ages of the participants, the average value is within the referenced normal values (47).

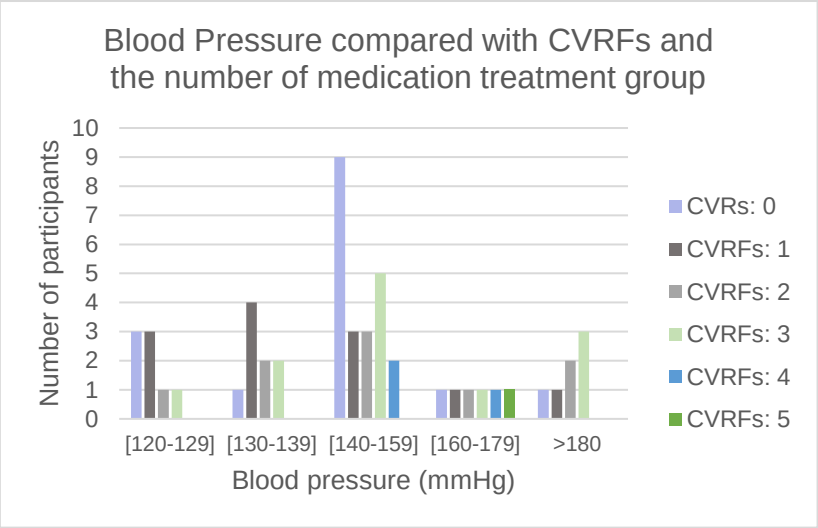



**Figure 47**: Blood pressure compared with CVRFs and the number of medication treatment group.

In the figure above, only blood pressure values above 130 mmHg were considered relevant since values below this value are considered normal in the literature (45). Risk factors only equal to or greater than 1 were also considered. We can see that in all blood pressure ranges there were patients with associated CVRFs, thus worsening hypertension states as seen in (45).

To study physiological parameters more accurately and better understand their influence on patients' anxiety levels throughout the exam, this parameter was correlated with the psychological parameters, STAI-S and VAS, and with the biochemical parameter, cortisol. The data are presented in Table 17.

**Table 17:** Statistic results to psychological, physiological, and biochemical parameters in Cross-sectional study.

| | | Sbp (mmHg) | Dbp (mmHg) | Beats/min | Breaths/min | _$SpO_2$% |
|---|---|---|---|---|---|---|
| **STAI-S** | Correlation Coefficient | **0.126** | **0.197** | **0.064** | **0.069** | **-0.134** |
| | Sig. (2-tailed) | 0.326 | 0.122 | 0.619 | 0.592 | 0.294 |
| | N | 63 | 63 | 63 | 63 | 63 |
| **VAS** | Correlation Coefficient | **0.005** | **0.049** | **0.007** | **0.143** | **-0.054** |
| | Sig. (2-tailed) | 0.966 | 0.704 | 0.958 | 0.265 | 0.675 |
| | N | 63 | 63 | 63 | 63 | 63 |
| **Cortisol** | Correlation Coefficient | **-0.093** | **-0.186** | **0.073** | **0.013** | **0.040** |
| | Sig. (2-tailed) | 0.526 | 0.202 | 0.620 | 0.930 | 0.785 |
| | N | 49 | 49 | 49 | 49 | 49 |

We observed from the table above that for both psychological and biochemical parameters, there was no statistically significant correlation, thus showing that physiological factors are not directly correlated with anxiety factors.

### 9.2.6 Image Quality

To analyse image quality parameters and the influence of anxiety on this, sociodemographic, psychological, biochemical, and physiological parameters were used as measurements.

1. Image Quality and sociodemographic parameters

All results were based on data from APPENDIX 5, APPENDIX 15 and APPENDIX 16.

To analyse the image quality of the Cross-sectional study, we first checked the number of patient movements according to socio-demographic parameters. We only use the parameters from previous studies. Due to the low sample number for patients with movements between 1-2, only the graph for the number of movements less than 1 pixel was presented (Figure 48).

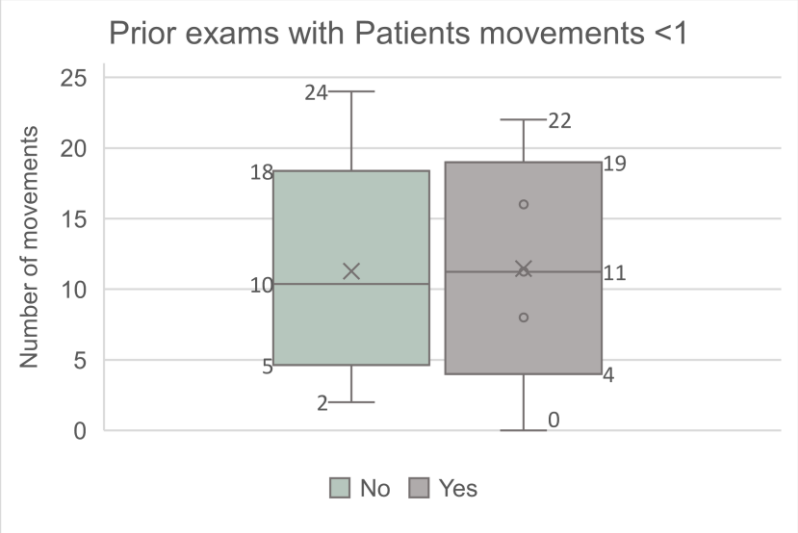



**Figure 48**: Participant´s previous imaging exams compared with number of movements <1.

We found that patients who had previously undergone imaging exams had a minimum number of movements of 0, an average of 11 and a maximum number of movements of 22. For patients who had never undergone medical imaging exams, the minimum value of movements was of 2, the average number of 11 and a maximum number of 24.

Although participants who had already taken the exam presented higher maximum values than the remaining participants, the average number of movements is the same for both groups.

## 2. Number of movements and psychological parameters

The results were based on data from APPENDIX 6,APPENDIX 8, APPENDIX 15 and APPENDIX 16.

Subsequently, we compared the number of movements of the patients with the psychological parameters STAI-S and VAS, for the T1 moment of the exam (Figure 49). The results were divided according to graphs for movements less than 1 pixel and between 1-2 pixels.

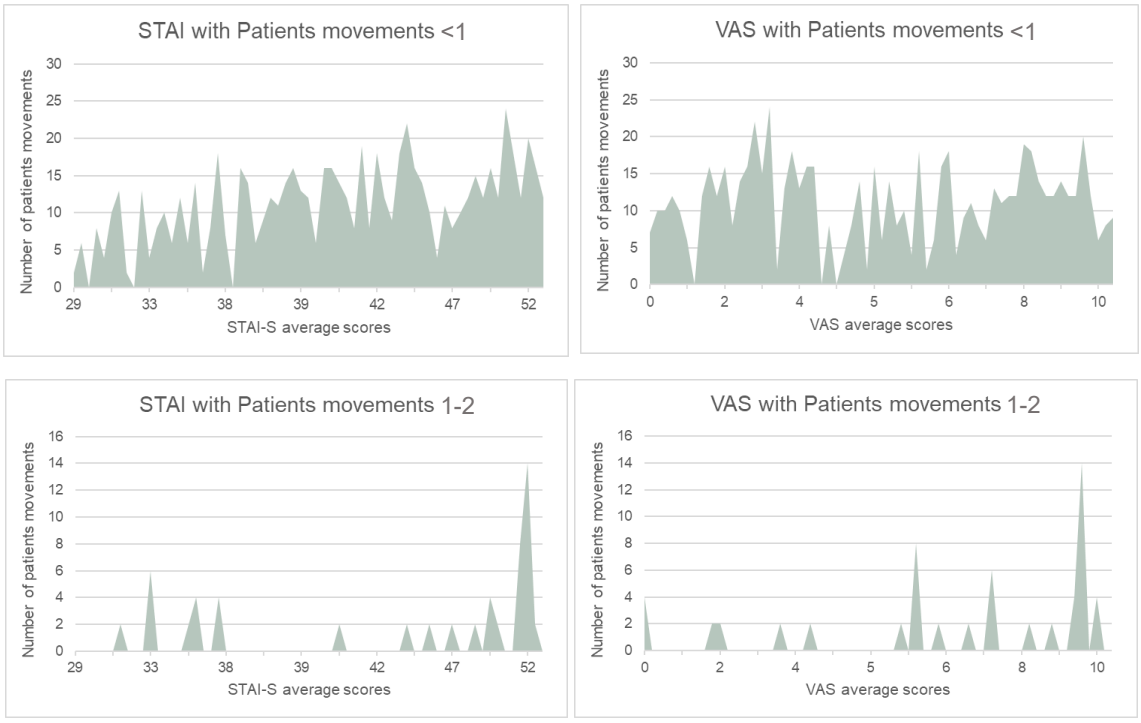



**Figure 49**: Patients movements <1 and between 1-2 compared with psychological parameters.

We found that, for patient movements of less than 0.5 pixel, although there is no statistical significance, the number of movements increases according to the STAI-S psychological parameter. In patient movements between 1-2 pixels, we also found that there is an increase in the number of patient movements with both psychological parameters.

## 3. Noise and psychological parameters

The results were based on data from APPENDIX 6,APPENDIX 8, APPENDIX 15 and APPENDIX 16. Next, we check the relationship between psychological parameters and image noise Figure 50.

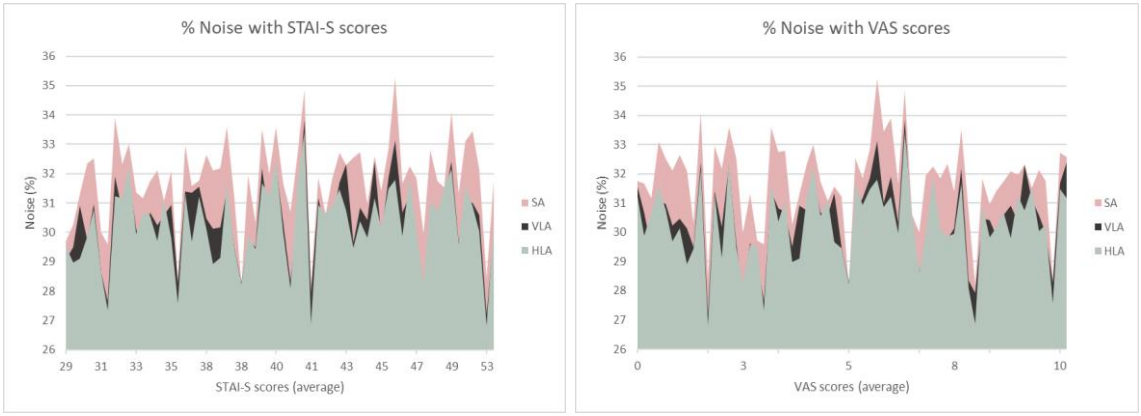



**Figure 50**: Comparison between Noise (%) and psychological parameters, for 3 heart projections, SA, VLA and HLA.

We verified that for both psychological parameters, the three projections (SA, VLA and HLA) presented similar results between them. However, comparing with the psychological parameter scores, we did not find a significant relationship between the increase in STAI-S and VAS with the increase in image noise.

For a better understanding of the results obtained for noise, the noise values in the reconstructed image should be lower than 33%. We checked the noise values of our results for each projection (SA, LVA and HLA). The results are presented in Figure 51.

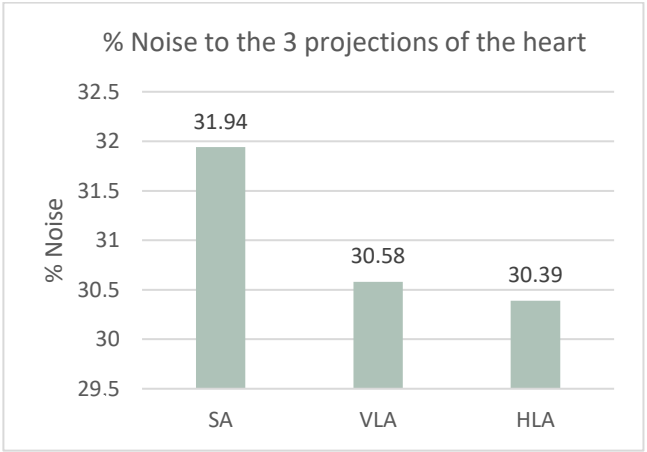



**Figure 51**: %Noise for the 3 different heart projections.

We note from the graph above those the mean noise values were 31.94% for SA, 30.58% for VLA and 30.39% for HLA. We found all values to be less than 33% and therefore, in agreement with the literature (116,117) our results for mean noise in the reconstructed images are considered acceptable.

## 4. Contrast and psychological parameters

The results were based on data from APPENDIX 6,APPENDIX 8, APPENDIX 15 and APPENDIX 16.

For contrast, we also checked its relationship with the biochemical parameters of the study. The results are shown in Figure 52.

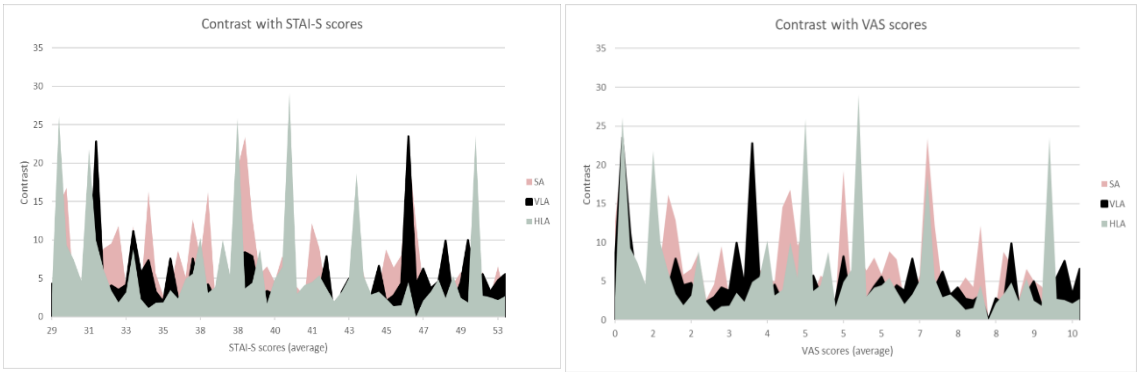



**Figure 52**: Correlation between contrast and psychological parameters (STAI-S and VAS) to 3 different heart projections, SA, VLA and HLA.

We verified that for both biochemical parameters, the three projections (SA, VLA and HLA) presented similar results between them. However, comparing with the psychological parameter scores, we did not find a significant relationship between the increase in STAI-S and VAS with the change in contrast in the images.

Since the biochemical parameters underwent many concentration changes throughout the examination due to their biochemical factors as well, these were not used in the image quality analysis. This was followed for the analysis of image quality with physiological parameters.

## 5. Image Quality and Physiological parameters

We then correlate image quality with physiological parameters (Table 18).

The results were based on data from APPENDIX 6,APPENDIX 8, APPENDIX 15 and APPENDIX 16.

**Table 18:** Statistic results to image quality compared with physiological parameters to Cross-sectional study.

| | | LHA: %Noise | LHA: Contrast | LVA: %Noise | LVA: Contrast | SA: %Noise | SA: Contrast | Nº of movement |
|---|---|---|---|---|---|---|---|---|
| **Sbp (mmHg)** | Correlation Coefficient | **0.009** | **0.067** | **0.078** | **0.120** | **0.132** | **0.082** | **0.118** |
| | Sig. (2-tailed) | 0.945 | 0.603 | 0.547 | 0.353 | 0.305 | 0.527 | 0.356 |
| | N | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| **Dbp (mmHg)** | Correlation Coefficient | **0.181** | **0.131** | **0.088** | **0.034** | **0.093** | **0.107** | **-0.020** |
| | Sig. (2-tailed) | 0.158 | 0.311 | 0.495 | 0.793 | 0.473 | 0.408 | 0.875 |
| | N | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| **Beats/min** | Correlation Coefficient | **.300*** | **0.151** | **.314*** | **0.063** | **.281*** | **0.174** | **-0.022** |
| | Sig. (2-tailed) | 0.119 | 0.240 | 0.013 | 0.624 | 0.027 | 0.177 | 0.864 |
| | N | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| **Breaths/min** | Correlation Coefficient | **0.013** | **0.176** | **0.060** | **0.140** | **0.072** | **0.150** | **0.182** |
| | Sig. (2-tailed) | 0.923 | 0.171 | 0.642 | 0.277 | 0.580 | 0.243 | 0.153 |
| | N | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| **$SpO_2$%** | Correlation Coefficient | **0.158** | **0.034** | **0.020** | **-0.145** | **0.064** | **0.051** | **-0.057** |
| | Sig. (2-tailed) | 0.220 | 0.793 | 0.875 | 0.262 | 0.623 | 0.692 | 0.656 |
| | N | 63 | 63 | 63 | 63 | 63 | 63 | 63 |

We observed from the table above that when correlating the physiological parameters with the image quality parameters we verified that there are statistically significant correlations between the heart rate and the noise image quality parameters and the average counts of this, for the 3 projections, SA, LVA and LHA.

## 9.3 Pilot Study

### 9.3.1 Measurement parameters

The Pilot study consists of a total of 34 participants. Data collection from participants was carried out in NM service 2 and NM service 3. In the first phase of the Pilot study, the sociodemographic and biochemical data of the sample of participants were analysed. In this study, the sample was collected in two different NM services. Figure 53 shows the distribution of patients in the two clinics. The results were based on data from APPENDIX 17.

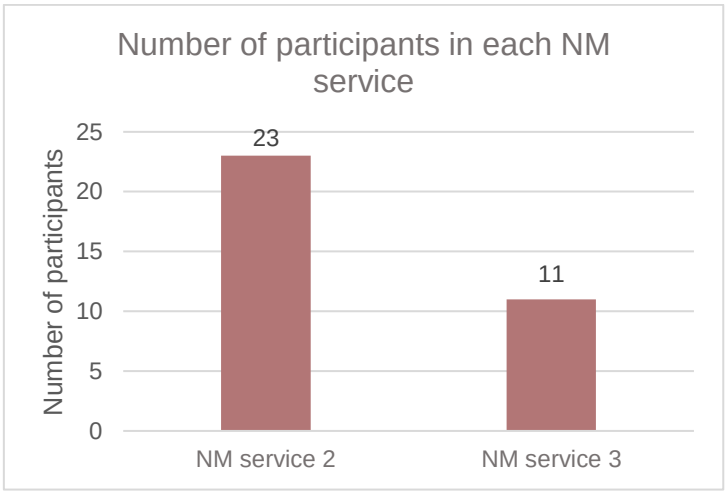



**Figure 53**: Participants distribution in NM services, for Pilot study.

The distribution of patient ages according to the gender of participants are represented in Figure 54.

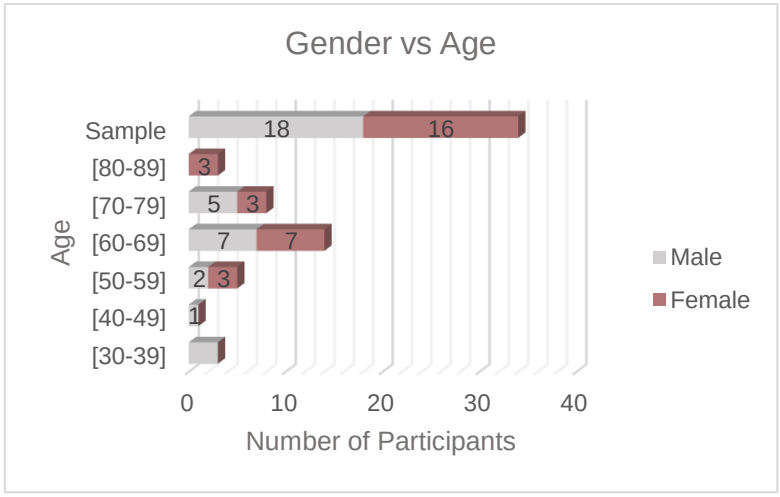



**Figure 54:** Participants gender distribution in different age groups.

We found that there are a total of 18 men and 16 women in the sample, the majority of whom are in the 60 to 69 age group.

Next, we analysed the sample according to biochemical parameters. The data on the different concentrations for the different exam times were taken from the APPENDIX 11 and can be observe in Figure 55.

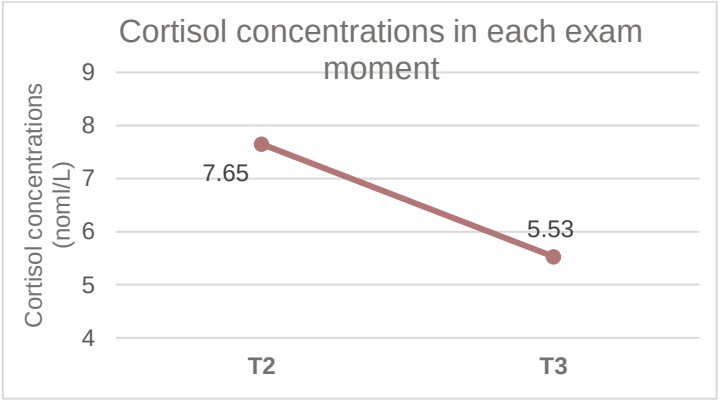



**Figure 55**: Cortisol concentrations in Pilot study sample to the two measurement moments in exam.

We found that the average cortisol concentration values for T2 were 7.65 nmol/L and for T3, 5.53 n/mol, thus there was a decrease in their concentrations throughout the rest phase of the exam.

### 9.3.2 Musical Intervention

The musical intervention used in this Pilot study was collected during the acquisition of resting images (1st images). Although the intervention procedure is the same for all participants, there are factors that are the participant's choice throughout this study. Patients had the option to choose their musical intervention from 5 possible options. The Figure 56 shows, for each option, the number of participants who chose it. The results were based on data from APPENDIX 17.

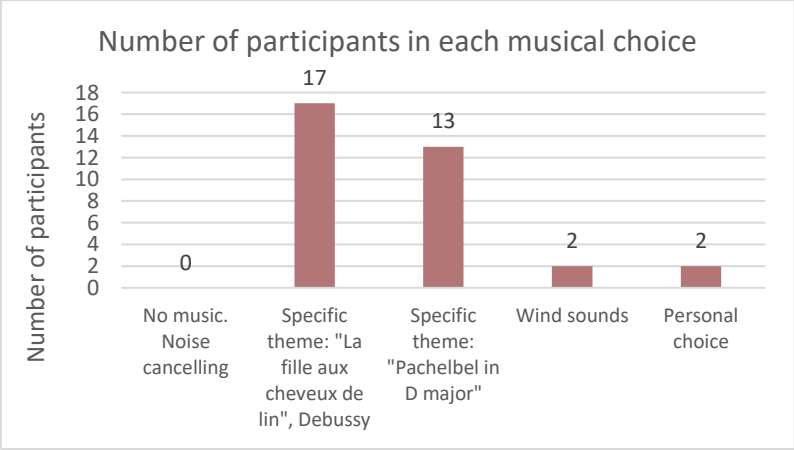



**Figure 56:** Different musical choices to compared with number of participants.

We can see from the figure above that the musical intervention most chosen by the participants was the specific theme "La fille aux cheveux de lin", a composition by Claude Debussy. The second most chosen intervention was the other specific musical theme “Pachelbel D major”. The intervention without music, just with headphones prepared for noise cancelling, was an option that had no participants. The following graph shows the choice of musical intervention by age and genre (Figure 57).

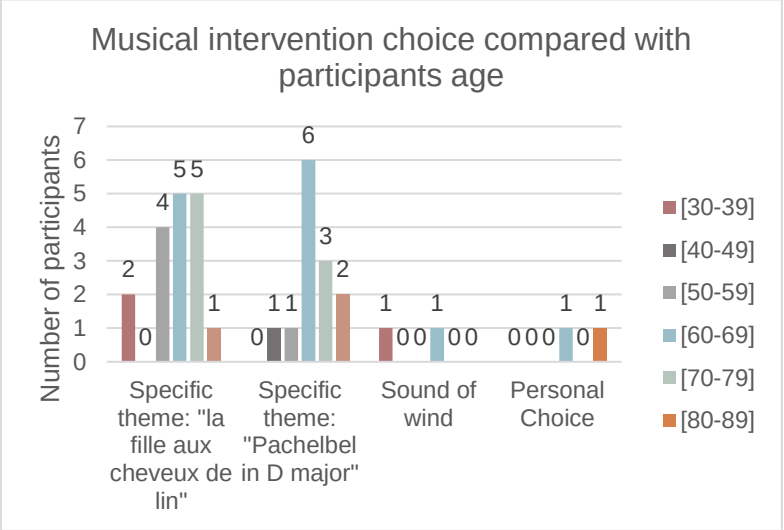



**Figure 57:** Musical choice in different participant´s age groups.

We can see in the figure above that for the specific theme “la fille aux cheveux de lin”, participants within the age range between 50 and 79 were the ones who most chose this theme. For the specific theme “Pachelbel D major”, participants aged between 60 and 69 chose this theme more. Participants belonging to the younger age group opted for sounds of wind or specific theme “la fille aux cheveux de lin”. Participants who made a musical choice were aged with 64 and 84 years old. In addition to the musical choice, volume is also a parameter that can be adjusted by participants. Figure 58 shows the volume chosen by the participants depending on their age.

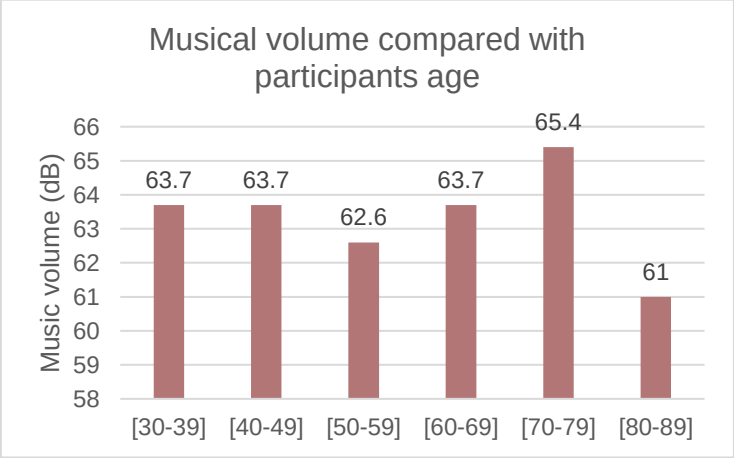



**Figure 58**: Musical volume compared with participant’s age.

For the volume chosen by participants depending on their age, we found that participants of all age groups chose volumes between 60-70 dB for the musical intervention.

Since the Pilot study was carried out in two different NM services, the range of services were not the same. The acquisition time in NM service 2 was 18 min and in NM service 3 it was 6 min. As such, we drew the graphics in Figure 59 that compares the acquisition times of the two services with the psychological parameters. The results were based on data from APPENDIX 17, APPENDIX 18 and APPENDIX 19.

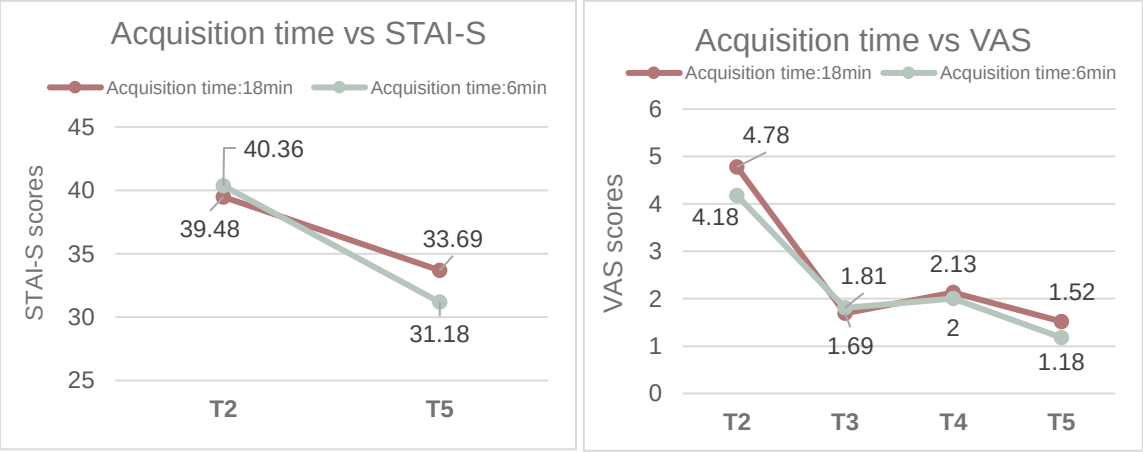



**Figure 59**: Comparison between psychological scores and different acquisition times.

In the figure above, we see that the scores of the two samples of participants at all times of the exam, both for the STAI-S and the VAS, were very similar to each other.

## 9.4 Cross-Sectional study and Pilot study comparations

For a comparative analysis between the Cross-sectional study and the Pilot study, psychological and physiological parameters were used, based on data from APPENDIX 17, APPENDIX 18, APPENDIX 19 and APPENDIX 21.

The following figure shows the comparison of the two studies using physiological parameters (Figure 60).

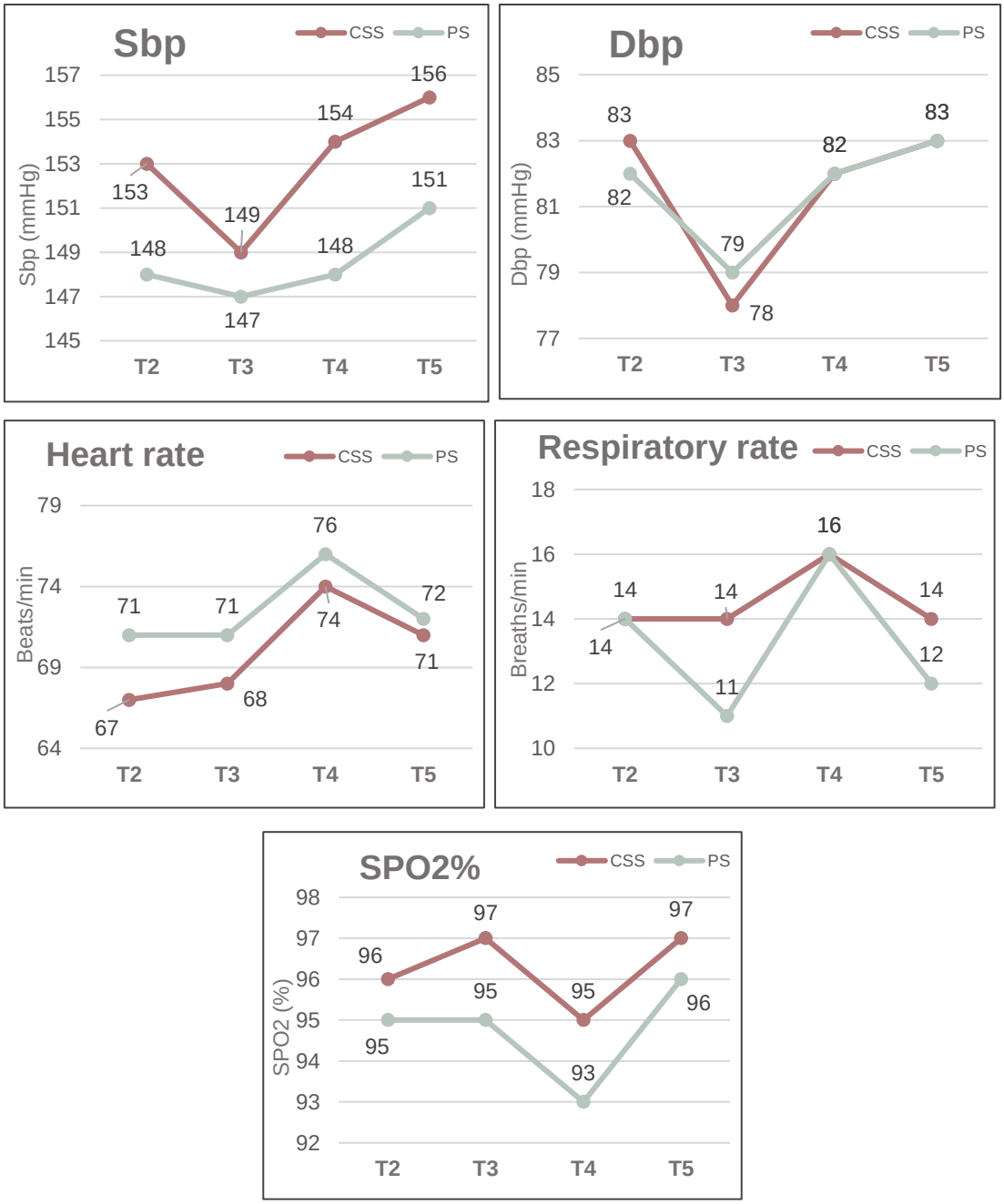



**Figure 60:** Comparison between physiological parameters from Cross-sectional study and Pilot study.

For the physiological parameters Blood Pressure, respiratory rate and $SpO_2$%, we verified that most of the physiological values, at each moment of the examination, are lower in the Pilot study compared to the Cross-sectional study sample. For the physiological parameter heart rate, the beats/min values at different moments of the exam were higher for the Pilot study sample compared to the Cross-sectional study sample.

The following figure shows the comparison of the two studies using the psychological parameters STAI-S and VAS (Figure 61).

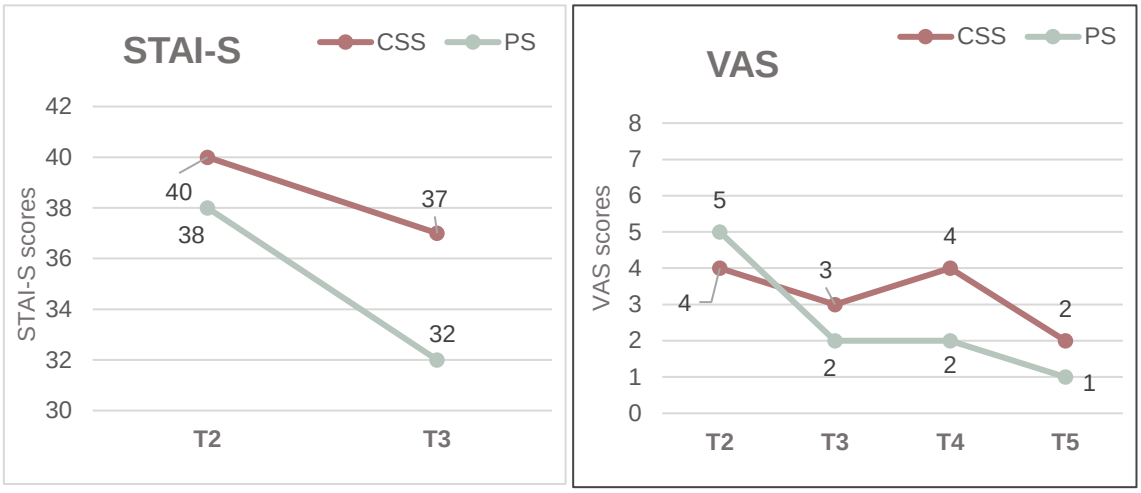



**Figure 61**: Comparison between psychological parameters from Cross-sectional study and Pilot study.

We verified that for both psychological parameters, the scores of both STAI-S and VAS are lower in the Pilot study sample than in the Cross-sectional study.

# 10 Discussion

For a clearer and more structured study of the project, it was divided into three phases of analysis: Analysis of the Cross-sectional study, analysis of the Pilot study and comparative analysis between the Cross-sectional study and the Pilot study. The total number of participants that was used for this study was n=97. The Cross-sectional study sample was collected in NM service 1 and had a total of n=63 participants, representing 64.9% of the total participants used in this project.

In a first phase, the sample of participants in the Cross-sectional study was analysed according to the participants' psychological, biochemical, physiological, and sociodemographic parameters, following the protocols used in the clinical practice of each service. Subsequently, the image quality was analysed, which was only analysed for this sample.

Regarding the first psychological parameter that was used, the STAI-S, we can see that it suffers a decrease in scores throughout the examination moments, with T1 having higher score values than T5.

Upon arrival at the hospital, factors such as lack of knowledge about the exam, arrival at the service, medical indication for the exam due to heart problems can cause higher levels of anxiety in the patient (13,14,135,226). We found that after T2 anxiety values decrease. During this phase of the examination, the radiopharmaceutical is administered to the patient, but also between these two moments the patient speaks to the doctor, and he explains again the entire procedure that he will carry out, clarifying possible doubts or concerns that the patient may have. Providing them pre-procedure information and clarifying to patients what they can expect during the examination can be an important factor in decreasing their anxiety (226). After the first images, anxiety levels decrease again. When the image acquisition is finished, the patient feels a sense of relief for having already taken the images (226). At T4 there is an increase in anxiety levels, compared to T3. When the patient finds out that they are going to take the second images of the exam, they may feel more anxiety again (226). Furthermore, at T4 patients undergo an exercise test. This peak in results may also be due to the physical effort induced in the patient, thus feeling greater anxiety or even confusing their anxiety with tiredness and physical exhaustion (227). In this way, it was possible to verify that the patient's anxiety decreased throughout the exam, which is in line with the expected results (226,227). When correlating the sociodemographic factors with the STAI scores for the participants in this sample, we found that when we compared the STAI-S scores with the gender of participants, we observed that females had higher scores and thus higher anxiety values than males, being consistent with other studies (228,229). When we compared the STAI-S scores according to whether the participants had previously undergone other imaging exams, we observed that there were no differences in the average score (both obtained an average score of 40) and, therefore, we conclude that in this sample, in the specific factor of performing imaging exams did not affect the anxiety that the patient felt.

Comparing the STAI-S scores according to the patients' educational qualifications, our results were in line with what was expected and what was observed in other studies since there were higher scores in patients with less education (average score of 45) and, according to the literature, patients' lack of knowledge may be associated with more specific fears and in turn, with higher levels of anxiety (13,14,135,226,230). Comparing the STAI-S scores according to the number of CVRFs for each patient, we observed that there was no decrease in scores as the number of risk factors is higher. There was an increase in scores for up to 2 risk factors and, therefore, an increase in patients' anxiety, but the same did not occur for patients with 3 risk factors and for 4 CVRFs as they suffered a decrease in scores, in other words, a decrease in your anxiety. A possible reason for this to have occurred is the fact that there are other factors and variables associated with the patient that can cause this type of changes, such as the age of the participant and the number of medications the patient takes, since the patient knowing who are being medicated tend to feel less anxiety about awareness of their health problems (123,226,231). This is the case of participants with 3 CVRFs. Despite showing a reduction in anxiety compared to patients with 2 CVRFs, they are the patients who presented a higher ratio of number of types of medication/patients, which may therefore be a supporting factor for having observed these results.

Regarding the second psychological parameter that was used, the VAS, we can see that it suffers a decrease in scores at different moments of the exam, with the highest values found at T1 (average score of 5) and the lowest scores at T5 (average score of 2), thus resulting in a reduction in the patient's anxiety, in line with the expected results. However, VAS scores showed an increase in values at T4 compared to T3. This may be justified by the fact that patients at this point in the exam perform the exercise test, which is associated with the stress phase of the exam. The patient as such may feel greater anxiety as he or she is performing a test that involves physical and mental effort, an effort that most patients may not be used to dealing with in their day-to-day lives and may even confuse at the moment with tiredness and exhaustion (149,151,232).

When correlating the sociodemographic factors with the VAS scores for the participants in this sample, we found that when we compare the VAS scores with the gender of participants, we observed that females have higher scores and thus higher anxiety values than males, just as was observed for STAI-S and in other studies in the literature (228,229). When we compared the VAS scores according to whether the participants had previously undergone other imaging exams, we observed that there were no differences in the average score (both obtained an average score of 5) and, therefore, we concluded that in this specific sample the factor of performing imaging exams did not affect the anxiety the patient felt. Comparing the VAS scores according to the patients' educational qualifications, we did not obtain the same results that were observed for the STAI-S. Participants without qualifications had a score equal to participants with up to secondary education (average score of 4) and the highest scores were found in participants with up to 9th grade education (average score of 5).

This difference in results can be explained by the fact that the VAS is a shorter and less accurate questionnaire in the assessment of anxiety like the STAI-S (145,148,151,233,234) and, therefore, the VAS scale is not as precise in the assessment of different parameters of anxiety that the patient may have when taking the questionnaire. Comparing the VAS scores according to the number of CVRFs of each patient, we observed that up to 1 risk factor there was an increase in VAS scores and, therefore, an increase in patients' anxiety, but then a decrease in values of scores was observed and in turn a decrease in patient anxiety, with lower anxiety values in patients with 4 risk factors (scores of 4). This can be justified by the variables of the participant's age and the number of medications the patient takes (235).

To guarantee these two measurements as being psychological parameters to evaluate the participants' anxiety levels, both were mutually correlated, for times T1 and T5. In this case, it was confirmed that for both T1 and T5 there is a statistically significant correlation between the two psychological parameters ($\rho$= .341 and $\rho$=.430, respectively). However, it must be considered that the two values are not close to 1 (strong significant correlation between two variables), that is, in the results between the two parameters there is a statistically significant correlation, but of low intensity (236). This weak intensity in its correlation may also be associated with the fact that the VAS, compared to the STAI-S, is a less accurate, precise, and detailed measure of anxiety (145,149,151).

Regarding the biochemical parameter that was used, the cortisol biomarker, we can see that it suffers a decrease in concentrations throughout the examination moments, with cortisol concentrations at T1 being higher than at T3. In this way, it was possible to verify that the patient's anxiety decreased throughout the examination also according to biochemical parameters. We also found that at time T0, despite the average cortisol concentrations being like T1, most participants had lower cortisol concentrations. The participant in time T0, which is carried out 2 days before the exam, is not yet in the hospital service to take the exam. This way, they may not yet feel the levels of anxiety that are directly related to the exam and its procedure (18,226,237).

Although the decrease in concentrations during the exam is in line with expectations, it is necessary to consider the fact that this biomarker changes during the day, being higher in the morning and decreasing throughout the day. The decrease in cortisol concentrations that we observed in our study could also be associated with this biochemical biomarker factor (24). In addition to this factor, sometimes patient appointments are delayed by services or are not made within the same timetable. These changes contribute to even more differences in cortisol concentrations (24). In this way, we also analysed how these service delays can influence variations in cortisol concentrations. We found that there are differences in cortisol concentrations for T1 and T2, but all measurements were carried out in the morning, so these cortisol variations are associated with other factors. For time T3, some appointments were already made in the afternoon, however the sample was low (only 7 participants), so we do not consider the concentrations of these participants to be statistically relevant.

When correlating sociodemographic factors with cortisol concentrations for participants in this sample, we found that when we compare concentrations with the gender of participants, males present higher scores and thus higher anxiety values than females. However, the average difference in concentrations is very close to each other (d=0.09), therefore, although the results do not agree with the results obtained in this study for psychological parameters, it is not a high difference in values to be considered significant. Comparing cortisol concentrations with participant´s age, it should be noted that all observed concentrations were lower than reference values in the literature, which correspond to a concentration of approximately 11 nmol/L for people under the age of 50 and approximately 12 nmol/L for people over 50 years of age. One of the reasons our sample has values lower than the reference concentrations is the fact that it was moved from services to college to be dosed. Even when complying with the appropriate transport conditions for the samples, there are unpredictable factors and timings that alter the sample integrity and may affect the degradation of this biomarker (238–241).

At the end of the analysis of biochemical parameters, these were correlated with the psychological parameters STAI-S and VAS. The results showed that no statistically significant results were obtained in both correlations (p=-0.187 and p=0.019, respectively). As shown above, cortisol is a biochemical parameter that has many variables and can therefore undergo many changes in its behaviour. Furthermore, the sample of participants used to measure biochemical parameters was reduced (n=47) due to the small amount of saliva we were able to collect from some participants. As such, there are few results to have statistically more impact.

Regarding physiological parameters, we found that, on average, blood pressure values at T1 were 153/86 mmHg, heart rate was 67 beats/min, breathing levels were 14 breaths/min and $SPO_2$% was 96%. We also verified that for physiological measurements blood pressure, heart rate and respiratory levels there is a peak in values at T4 and for $SPO_2$% a marked decrease in values. As observed in other previous parameters, this phenomenon is associated with the effort test that participants perform in the stress phase of the exam. For this same reason, the results obtained showed higher values (and lower values for $SPO_2$%) at T5 than at T1. As such, according to the literature, in the average value of the first 3 moments of the study, we found that the patients' blood pressure is classified as “Hypertension Grade Sbp: 140-159 mmHg and/or Dbp 90-99 mmHg” (45), patients' respiratory rate is on average 14 breaths/min, values slightly below normal reference values (48), and $SPO_2$% is at average values classified as insufficient (50). Some physiological parameters are also dependent on the patient's sociodemographic factors, such as blood pressure due to the patient's number of risk factors and heart rate due to the age of the participants. By correlating the physiological parameters with these parameters we can verify that the heart rate has an average in the sample of 68.05 beats/min, a minimum of 42 beats/min and a maximum of 101 beats/min. Comparing with literature values, we observed that in the sample, for different ages of participants, the average value is within the referenced normal values (47). In the blood pressure factor with the number of CVRFs for each participant, we found that in all ranges of blood pressure measurements there were patients with associated CVRFs, thus worsening the states of hypertension as seen in (45).

Correlating the physiological parameters with the psychological parameters STAI-S and VAS, we found that for both the psychological parameters and the biochemical parameters, there was no statistically significant correlation (Table 16). High levels of state anxiety indicate high levels of anxiety at the time of assessment (242). The anxious reaction contains physiological, emotional, and cognitive components (138). In the case of this study, it was concluded that physiological parameters may not be directly related to the levels of anxiety that patients feel during these exams since, contrary to other medical imaging exams, MPS has associated physical effort on the part of the patient.

To finalize the analysis of the Cross-sectional study, the image quality parameters, and the influence of anxiety on this were analysed. To this end, sociodemographic, psychological, biochemical, and physiological parameters were used. According to the literature, high levels of patient anxiety cause involuntary and voluntary movements in patients. These, in turn, cause artifacts in the images and make diagnostic tests difficult (13,14,25,169).

In a first analysis, we checked the number of movements that patients had during the exam. In our study we classify movements < 1 in statistically significant and statistically significant between 1-2 pixels. When we compared the number of movements with socio-demographic parameters, we found that patients who had already undergone medical imaging exams had higher maximum movement values (maximum number of movements = 24) than patients who had never undergone an exam (maximum number of movements = 22), however the average value of movements was the same for both groups (number of movements =11). Therefore, it was not possible to conclude in the analysis of image quality whether previous examinations had an influence on the number of movements of the patient and, therefore, on the patient's anxiety.

When we compared the number of movements with the psychological parameters, we found that movements of less than 1 pixel, despite not being statistically significant, showed a significant correlation with the STAI-S scores. The same did not occur for the VAS, however, this may be because this parameter is not as rigorous an analysis of anxiety as the STAI-S. With a number of movements between 1-2 pixels, there was a lower number of samples to be analysed, however, we found for both biochemical parameters that there was an increase in the number of movements with the increase in scores. In this way, we found that the number of movements increases depending on the patient's anxiety when performing the exam, which is in line with the expected results (13,14,25,169). Biochemical factors were not used to analyse image quality since their values were much lower than the reference values and their changes throughout the day could affect the results.

We subsequently checked the relationship between these biochemical parameters and other factors related to image quality: average noise (%) and contrast. For the % noise of the three projections analysed (SA, VLA and HLA) we verified that when comparing with the psychological parameters of the study, no significant differences were found with the increase in patients' anxiety. The same occurred for image contrast.

Although image motion artefacts, such as % noise and contrast, are also related to patient movement during image acquisition in these exams and in turn with the decrease in image quality, there are other factors not related to the patient which can also manipulate the alteration of these two image quality parameters, as is the case where noise comes from the random nature of the radioactive decay process, which causes statistical variations in the rate of observe noise, and contrast also depends on the choice of radiopharmaceutical used in the exams (110–112,115,119).

For the specific case of %noise, to be clinically meaningful, the noise in the reconstructed image must be less than 33% (116,117), which was observed in our results where the % noise for SA, VLA and HLA were 31.94%, 30.58% and 30.39%, respectively. Therefore, in our study, this parameter had values considered acceptable for observing changes in image quality. When we analysed the image quality factors with the physiological parameters, we found that there were significant correlations between the % noise with the physiological parameter heart rate, in the SA projection (p=.281), LVA (p=.314) and LHA (p=.300). Therefore, despite this being a statistically significant correlation of weak intensity, we can conclude that the heart rate had an influence on the quality of the images in our study. For the remaining physiological parameters, we did not find statistically significant correlations (Table 18).

In addition to the reasons presented above and, despite our sample presenting lower score values at the end of the exam compared to the scores at the beginning of the exam, these values are not considered very high on the anxiety scale, both for the STAI-S and for the VAS, since on the STAI-S scale the average score at T1 was 41.3, considered a “moderate-anxiety” level and for VAS the average score at T1 was 5, considered “Mild-moderate” anxiety (142,150,151). Therefore, patients in general in the Cross-sectional study did not have high levels of anxiety. This may justify the results obtained in the quality image, which did not suffer significant differences in the face of different anxieties of the patients in the studied sample.

In a second phase, the sample of participants in the Pilot study was analysed according to the sociodemographic parameters of the sample and the parameters of the musical intervention. psychological, biochemical, physiological, and sociodemographic characteristics of the participants, following the protocols used in the clinical practice of each service. The Pilot study sample was collected in two different NM services, obtaining a total of n=34 participants (35.1% of the total project sample). In NM service 2 we collected a total of n=23 participants and in NM service 3 a total of n=11 participants.

Regarding the sociodemographic parameters of the patients, we can see that the sample has a greater number of male participants, and most participants in this study are in the age group of 60 to 69 years old. The musical intervention used in this Pilot study was applied only during the acquisition of resting images (1st images), so that there would be no interference from changes to the patients resulting from the exercise test induced in the stress phase. In analysing the characteristics of the musical intervention, we found that the specific musical themes “La fille aux cheveux de lin”, Debussy and “Pachelbel D major” were the participants' preferred choices.

Both themes belong to the classical musical genre. This musical genre, according to other studies, is a genre that causes relaxation and harmonious beats (197,243). The specific theme “Pachelbel D major” was previously associated with lower stress levels, blood pressure, pulse, and body temperature (222). Furthermore, classical music is a genre that mostly uses frequencies between 60-80 beats. Thus, current findings indicates that music around 60-80 beats per minute can cause synchronization between the brain and the breath, causing alpha brainwaves, which are present when we are relaxed and conscious (244). For the volume chosen by participants depending on their age, we found that participants of all age groups chose, on average, a volume of 60 dB for the musical intervention. These values are within reference values used in other sound interventions (174).

Regarding the biochemical parameters of the study, we found that these were not always recorded at the same moment in the process. Due to changes and impossibilities of services in the Pilot study sample, cortisol was measured only at times T2 and T3, while in the Cross-sectional study sample it was at times T0, T1, T2 and T3. As it is a biomarker that changes its concentrations throughout the day (158,162,242) and there was a large discrepancy in data collected from the Cross-sectional study group to the Pilot group, this was only analysed individually for each study and was not used for comparative results between studies. We therefore verified for the Pilot study sample that there was a decrease in cortisol concentrations from moment T2 to moment T3, thus indicating a possible decrease in the patient's anxiety levels.

Since the Pilot study was carried out in two different NM services, the gamma cameras of the services were not the same and, therefore, the resting image acquisition time was different, taking a total of 18 min in NM service 2 and 6 min in NM service 3. This way we analysed whether acquisition time was an influencing factor on patients' anxiety. We verified through the psychological parameters STAI-S and VAS that the scores of the two samples of participants for all moments of the exam were very similar to each other, thus concluding that the acquisition time was not a factor influencing the patients' anxiety.

In a third and final phase of the project, the results obtained in the Cross-sectional study were compared with the Pilot study, using psychological, biochemical, and physiological parameters.

When comparing the physiological parameters of the two studies, we found that for the physiological parameters blood pressure, respiratory rate and $SpO_2$%, the Pilot study sample presented lower values than the Cross-sectional study sample. For heart rate, higher values were observed in the Pilot study sample. In this parameter it was expected that the beats/min values would be lower in the SP sample and for the $SpO_2$% parameter it was expected that the values would be higher. These differences may have occurred since the sample of the Pilot study was smaller than the sample of the Cross-sectional study, and therefore the sample of results is insufficient to draw further conclusions.

Regarding the psychological parameters of the study, we found that these were not always recorded at the same moment in the process. Due to changes and impossibilities of services in

collecting the Pilot study sample, STAI-S was measured at times T2 and T5, while in the Cross-sectional study sample it was at times T1 and T5. Despite these being different times, as this is a flexible psychological parameter, and the collection times were carried out at similar times, comparisons of these parameters were carried out between studies.

When comparing the psychological parameters STAI-S and VAS in the two studies, we found that the participants in the Pilot study presented STAI-S and VAS scores lower than the scores obtained for participants in the Cross-sectional study, at all times of measurement of the exam. For biochemical parameters, the same was always verified, with lower concentration values for the Pilot study sample. Thus, we found that with lower scores for psychological parameters and lower concentrations of the biochemical parameter, cortisol, the anxiety of participants in the Pilot study was lower than the anxiety of the Cross-sectional study sample, in line with previous studies (134,135,176,182,213,222,245). Therefore, we conclude that musical intervention has an influence on patients' anxiety when they undergo MPS exams.

# 11 Conclusions

Remaining that the main objective of the study was to evaluate the impact of patient's anxiety on the quality of myocardial perfusion scintigraphy images, it was confirmed that there was an influence of anxiety on image quality, but only in some qualitative parameters. The image quality underwent significant changes in the noise % parameters, with a statistically significant weak intensity correlation being observed between these two parameters and the physiological heart rate parameter. Furthermore, it was found that the number of patient movements during image acquisition increases with the scores of the psychological parameter STAI-S, showing a positive correlation between the two factors, proving that anxiety in this study had an influence on this quality-of-life parameter image.

To achieve the main objective, several intermediate objectives were met through the analysis of psychological, biochemical, physiological, and sociodemographic parameters of the sample. In the Cross-sectional study sample, we mostly concluded that parameters such as age, gender and educational qualifications had an influence on patients' anxiety. The analysis of biochemical parameters, despite being an asset for more detailed studies in general the results were not clear since their concentrations change over time throughout the day and can therefore be a manipulative factor in the stud. Regarding the patients' physiological parameters, these also underwent many changes in values during the examination due to the exercise test associated with the stress phase, thus concluding that the physiological parameters may not be directly related to the levels of anxiety that patients feel during MPS exams.

In the Pilot study, carried out after the Cross-sectional study, which used a musical intervention, we found that the specific musical themes "La fille aux cheveux de lin", Debussy and "Pachelbel D major" were the most chosen by the participants, with an average volume of 60dB. Regarding the biochemical parameters of the participants, we observe a decrease in values between the two times of exam. Despite meeting expectations, the results, like in Cross-sectional study, can be affected by concentrations throughout the day.

When comparing the results of the Cross-sectional study with the Pilot study, a decrease in the psychological was noted. Regarding physiological parameters, it was not possible to draw concrete conclusions about their changes. Unlike other types of medical imaging exams, the myocardial perfusion scintigraphy exam presents an associated stress test for its performance. As this specific study collected data only for patients who underwent the 1-day MPS protocol, all data from our sample is taken from the same day of examination. In this way, there is a greater influence of these tests, especially on the participant's physiological parameters, which may deviate from the expected results.

Thus, we can conclude that physiological parameters such as heart rate have an influence on image quality and the number of patients' movements is influenced by their anxiety during CPM exams.

# 12 Limitations of study

During the experimental execution of this work, and considering the results achieved, it was clear that there were certain limitations inherent to the applied methodology, both due to time restrictions and protocol changes. Therefore, the main limitations of the study carried out and some suggestions for improvement for future work are presented below.

Regarding the implementation of the project, it was started in the NM service of the Hospital Particular de Almada. When the project was launched, it was intended to be carried out entirely at HPA, however, halfway through the project, the service suffered some constraints and was forced to close, meaning it was necessary to look for a new service to continue the study. In addition to time lost in this search process, the change of services meant that some moments of the protocol were lost as its implementation would delay the schedules of other services.

Data collection for image quality analysis, due to HPA service closing, had to be “accelerated” since the service would later destroy the participants' data and their processing could only be done at the HPA. Therefore, although we collected all the data we intended to evaluate, if the treatment had not been limited in time, we could have verified other data that we considered relevant to our study. Regarding the evaluation of our sample, we found that there was a very complete study in terms of the parameters used for its analysis. However, the project sample, both the Cross-sectional study and the Pilot study, is small. Some data were not possible to evaluate in their entirety as being statistically significant due to the lack of participants to reach more significant results. The present study used some measurement parameters, in the case of physiological parameters that vary greatly with factors associated with the patient and factors associated with this specific exam, since it has an associated exercise test. In the case of the biochemical parameter cortisol, despite being a biomarker of anxiety, its concentrations vary throughout the day and, therefore, become a variable that influences our results.

In the Pilot study, positive results were found regarding the impact of the musical intervention on the patient's experience and, in turn, in reducing patients' anxiety levels during MPS exams. However, it is still not clear in the literature which are the best musical intervention approaches to be carried out in medical imaging exams.

Therefore, considering the limitations mentioned above, it is thought that, in the future, it will be pertinent:

- Restructure the protocol in MPS exams and verify the type of parameters to be used for their analysis;
- Develop studies with a greater number of participants, in order to enable statistically significant results;
- Seek to research musical methods and interventions in medical imaging exams, to improve the patient experience and continue to develop more studies with musical intervention in MPS exams;

# 13 References


1. World Health Organization. Cardiovascular Diseases. Vol. 252, JAMA: The Journal of the American Medical Association. 2021. p. 2177–80.

2. Nissen L, Winther S, Westra J, Ejlersen JA, Isaksen C, Rossi A, et al. Diagnosing coronary artery disease after a positive coronary computed tomography angiography: The Dan-NICAD open label, parallel, head to head, randomized controlled diagnostic accuracy trial of cardiovascular magnetic resonance and myocardial perfusion s. Eur Heart J Cardiovasc Imaging. 2018;19(4):369–77.

3. Underwood SR, Anagnostopoulos C, Cerqueira M, Ell PJ, Flint EJ, Harbinson M, et al. Myocardial perfusion scintigraphy: The evidence - A consensus conference organised by the British Cardiac Society, the British Nuclear Cardiology Society and the British Nuclear Medicine Society, endorsed by the Royal College of Physicians of London and t. Eur J Nucl Med Mol Imaging. 2004;31(2):261–91.

4. Henzlova MJ, Duvall WL, Einstein AJ, Travin MI, Verberne HJ. ASNC imaging guidelines for SPECT nuclear cardiology procedures: Stress, protocols, and tracers. J Nucl Cardiol. 2016;23(3):606–39.

5. Sanz M, Marco del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, et al. Periodontitis and cardiovascular diseases: Consensus report. J Clin Periodontol. 2020;47(3):268–88.

6. Düsing P, Heinrich NN, Al-Kassou B, Gutbrod K, Dörmann P, Nickenig G, et al. Analysis of circulating ceramides and hexosylceramides in patients with coronary artery disease and type II diabetes mellitus. BMC Cardiovasc Disord. 2023;23(1):4–9.

7. Farley A, McLafferty E, Hendry C. The cardiovascular system. Nurs Stand. 2012;27(9):35–9.

8. Desai MY, Windecker S, Lancellotti P, Bax JJ, Griffin BP, Cahlon O, et al. Prevention, Diagnosis, and Management of Radiation-Associated Cardiac Disease: JACC Scientific Expert Panel. J Am Coll Cardiol. 2019;74(7):905–27.

9. Eurostat. Cardiovascular diseases statistics - Statistics Explained. Eurostat: statistics explained. 2015. p. 1–14.

10. Vieira L. Contributo Para a Optimização Dos Estudos De Perfusão Miocárdica Utilizando Imagens De Medicina Nuclear Sincronizadas Com O Electrocardiograma [Internet]. Vol. I. 2010. Available from: https://repositorio.ul.pt/bitstream/10451/4500/1/ulsd061408_td_Lina_Vieira.pdf

11. Vieira L, Faria D, Patrina J, Nunes C, Sousa D, Ribeiro L, et al. Estudo da influência do número de ciclos por projecção no cálculo da fracção de ejecção ventricular esquerda em estudos Gated-SPECT. Saúde Tecnol. 2008;45–55.

12. Yoon JH, Nickel MD, Peeters JM, Lee JM. Rapid imaging: Recent advances in abdominal MRI for reducing acquisition time and its clinical applications. Korean J Radiol. 2019;20(12):1597–615.

13. Grilo A, Vieira L, Carolino E, Oliveira C, Pacheco C, Castro M, et al. Anxiety in Cancer Patients during 18 F-FDG PET/CT Low Dose: A Comparison of Anxiety Levels before and after Imaging Studies . Nurs Res Pract. 2017;2017:1–9.

14. Vieira L, Pires A, Grilo A. Anxiety experienced by oncological patients who undergo 18F-FDG PET CT: A systematic review. Radiography [Internet]. 2021;27(4):1203–10. Available from: https://doi.org/10.1016/j.radi.2021.06.001

15. Basso D, Passmore G, Holman M, Rogers W, Walters L, Zecchin T, et al. Semiqualitative visual and quantitative morphometric evaluations of reduced scan time and wide-beam reconstruction in rest-gated stress SPECT myocardial perfusion imaging. J Nucl Med

Technol. 2009;37(4):233–9.

16. Wheat JM, Currie GM. Impact of patient motion on myocardial perfusion SPECT diagnostic integrity: Part 2. J Nucl Med Technol. 2004;32(3):158–63.

17. King S, Woodley J, Walsh N. A systematic review of non-pharmacologic interventions to reduce anxiety in adults in advance of diagnostic imaging procedures. Radiography [Internet]. 2021;27(2):688–97. Available from: https://doi.org/10.1016/j.radi.2020.09.018

18. Munn Z, Jordan Z. Interventions to reduce anxiety, distress and the need for sedation in adult patients undergoing magnetic resonance imaging: A systematic review. Int J Evid Based Healthc. 2013;11(4):265–74.

19. Munn Z, Jordan Z. The effectiveness of nonpharmacologic interventions to reduce anxiety and increase patient satisfaction and comfort during nuclear medicine imaging. J Med Imaging Radiat Sci [Internet]. 2014;45(1):47–54. Available from: http://dx.doi.org/10.1016/j.jmir.2013.10.006

20. Munn Z, Jordan Z. The effectiveness of interventions to reduce anxiety, claustrophobia, sedation and non-completion rates of patients undergoing high technology medical imaging. JBI Libr Syst Rev. 2012;10(19):1122–85.

21. Cheetu S, Medeiros M, Winemaker L, Li M, Bartel L, Foster B, et al. Understanding the Effects of Music Care on the Lived Experience of Isolation and Loneliness in Long-Term Care: A Qualitative Study. Healthc. 2022;10(3).

22. de Witte M, Spruit A, van Hooren S, Moonen X, Stams GJ. Effects of music interventions on stress-related outcomes: a systematic review and two meta-analyses. Health Psychol Rev [Internet]. 2020;14(2):294–324. Available from: https://doi.org/10.1080/17437199.2019.1627897

23. Tan DJA, Polascik BA, Kee HM, Hui Lee AC, Sultana R, Kwan M, et al. The Effect of Perioperative Music Listening on Patient Satisfaction, Anxiety, and Depression: A Quasiexperimental Study. Anesthesiol Res Pract. 2020;2020.

24. Larsson CA, Gullberg B, Råstam L, Lindblad U. Salivary cortisol differs with age and sex and shows inverse associations with WHR in Swedish women: A cross-sectional study. BMC Endocr Disord. 2009;9:1–11.

25. Abreu C, Grilo A, Lucena F, Carolino E. Oncological Patient Anxiety in Imaging Studies: the PET/CT Example. J Cancer Educ [Internet]. 2017;32(4):820–6. Available from: http://dx.doi.org/10.1007/s13187-016-1069-3

26. Zahraei-Moghaddam SM, Haghighatafshar M, Shekoohi-Shooli F, Miladi S, Farhoudi F. Toward applying a device to reduce motion artifact during imaging: a randomized controlled trial. Expert Rev Med Devices [Internet]. 2022;19(2):189–94. Available from: https://doi.org/10.1080/17434440.2022.2035215

27. Eysenck MW, Derakshan N, Santos R, Calvo MG. Anxiety and cognitive performance: Attentional control theory. Emotion. 2007;7(2):336–53.

28. Pass my exams. The Heart [Internet]. 2014. Available from: http://www.passmyexams.co.uk/GCSE/biology/enzymes-and-digestion.html

29. Miller LM, Gal A. Cardiovascular System and Lymphatic Vessels. Pathol Basis Vet Dis Expert Consult. 2017;561-616.e1.

30. Lecturi Medical. Heart Anatomy. 2015.

31. Park DS, Fishman GI. Development and function of the cardiac conduction system in health and disease. J Cardiovasc Dev Dis. 2017;4(2):1–16.

32. Arshad A, Atkinson AJ. A 21st century view of the anatomy of the cardiac conduction system. Transl Res Anat [Internet]. 2022;28(April):100204. Available from: https://doi.org/10.1016/j.tria.2022.100204

33. Fathala A. Myocardial Perfusion Scintigraphy: Techniques, Interpretation, Indications and Reporting. Ann Saudi Med. 2011;31(6):625–34.

34. Mori S, Spicer DE, Anderson RH. Revisiting the anatomy of the living heart. Circ J. 2015;80(1):24–33.

35. Ideker RE, Kong W, Pogwizd S. Purkinje fibers and arrhythmias. PACE - Pacing Clin Electrophysiol. 2009;32(3):283–5.

36. Filipe P, Castro A De, Gatehouse P, Firmin PD. FIRST-PASS MYOCARDIAL PERFUSION MRI: ARTIFACTS AND ADVANCES. 2010;

37. Buckberg GD, Nanda NC, Nguyen C, Kocica MJ. What is the heart? Anatomy, function, pathophysiology, and misconceptions. J Cardiovasc Dev Dis. 2018;5(2).

38. Robinson WF, Robinson NA. Cardiovascular System [Internet]. Sixth Edit. Vol. 3, Jubb, Kennedy and Palmer's Pathology of Domestic Animals: Sixth Edition. Elsevier Inc.; 2016. 1-101.e1 p. Available from: http://dx.doi.org/10.1016/B978-0-7020-5319-1.00012-8

39. Excitation C. Cardiac Electrophysiology and Electrocardiogram Interpretation. Structure. 2010. p. 1–17.

40. Sullivan BA, Fairchild KD. Vital signs as physiomarkers of neonatal sepsis. Pediatr Res [Internet]. 2022;91(2):273–82. Available from: http://dx.doi.org/10.1038/s41390-021-01709-x

41. Mok WQ, Wang W, Liaw SY. Vital signs monitoring to detect patient deterioration: An integrative literature review. Int J Nurs Pract. 2015;21(S2):91–8.

42. Hopskins J. Vital Signs (Body Temperature, Pulse Rate, Respiration Rate, Blood Pressure) | Johns Hopkins Medicine [Internet]. 2020. Available from: https://www.hopkinsmedicine.org/health/conditions-and-diseases/vital-signs-body-temperature-pulse-rate-respiration-rate-blood-pressure

43. Flack JM, Adekola B. Blood pressure and the new ACC/AHA hypertension guidelines. Trends Cardiovasc Med [Internet]. 2020;30(3):160–4. Available from: https://doi.org/10.1016/j.tcm.2019.05.003

44. Schutte AE, Kollias A, Stergiou GS. Blood pressure and its variability: classic and novel measurement techniques. Nat Rev Cardiol. 2022;19(10):643–54.

45. European Society of Cardiology. Definition of hypertension and pressure goals during treatment (ESC-ESH Guidelines 2018) [Internet]. Vol. 17, E-Journal-of-Cardiology-Practice. 2019. p. 1–12. Available from: https://www.escardio.org/Journals/E-Journal-of-Cardiology-Practice/Volume-17/definition-of-hypertension-and-pressure-goals-during-treatment-esc-esh-guidelin

46. Cygankiewicz I, Zareba W. Heart rate variability [Internet]. 1st ed. Vol. 117, Handbook of Clinical Neurology. Elsevier B.V.; 2013. 379–393 p. Available from: http://dx.doi.org/10.1016/B978-0-444-53491-0.00031-6

47. Surgical and Skin Institute. What your eyes can tell you about your health | Best Health Magazine Canada [Internet]. 2017. Available from: http://www.besthealthmag.ca/best-you/eye-health/what-your-eyes-can-tell-you-about-your-health/

48. Mohamad Hadis NS, Amirnazarullah MN, Jafri MM, Abdullah S. IoT Based Patient Monitoring System using Sensors to Detect, Analyse and Monitor Two Primary Vital Signs. J Phys Conf Ser. 2020;1535(1).

49. Fleming S, Thompson M, Stevens R, Heneghan C, Plüddemann A, MacOnochie I, et al. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: A systematic review of observational studies. Lancet [Internet]. 2011;377(9770):1011–8. Available from: http://dx.doi.org/10.1016/S0140-6736(10)62226-X

50. Schade M. Oxygen saturation: normal values & measurement - cosinuss [Internet]. Cosinuss°. 2021. Available from: thermometers: How to choose the right tool for the job

51. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. Heart disease and stroke statistics - 2018 update: A report from the American Heart Association. Vol. 137, Circulation. 2018. 67–492 p.

52. Assmann G, Carmena R, Cullen P, Fruchart JC, Jossa F, Lewis B, et al. Coronary heart disease: Reducing the risk: A worldwide view. Circulation. 1999;100(18):1930–8.

53. Ahmadi M, Lanphear B. The impact of clinical and population strategies on coronary heart disease mortality: an assessment of Rose's big idea. BMC Public Health [Internet]. 2022;22(1):1–9. Available from: https://doi.org/10.1186/s12889-021-12421-0

54. Strauss HW, Douglas Miller D, Wittry MD, Cerqueira MD, Garcia E V, Iskandrian AS, et al. PROCEDURE GUIDELINES Procedure Guideline for Myocardial Perfusion Imaging PART I:PURPOSE. J NucÃ-Med. 1998;39(5):918–23.

55. (CAD) CAD. Coronary Artery Disease. Center of Disease Control and Prevention. 2019.

56. Harris KC, Rankin K, Aminzadah B, Ross H, Andrew G. Modifiable cardiovascular risk factors in adolescents and adults with congenital heart disease. 2018;(January):1–8.

57. Moon JR, Song J, Huh J, Kang I, Park SW, Chang S, et al. Analysis of Cardiovascular Risk Factors in Adults with Congenital Heart Disease. 2015;416–23.

58. Levene J, Cambron C, Mcgrath L, Colina I, Broberg C, Ramsey K, et al. International Journal of Cardiology Congenital Heart Disease Prevalence of traditional and non-traditional cardiovascular risk factors in adults with congenital heart disease. Int J Cardiol Congenit Hear Dis [Internet]. 2023;11(October 2022):100424. Available from: https://doi.org/10.1016/j.ijcchd.2022.100424

59. Shah A, Gandhi D, Srivastava S, Shah KJ, Mansukhani R. Heart failure: A class review of pharmacotherapy. P T. 2017;42(7):464–72.

60. Rossello X, Pocock SJ, Julian DG. Long-term use of cardiovascular drugs challenges for research and for patient care. J Am Coll Cardiol. 2015;66(11):1273–85.

61. Lindsay Slowiczek P. Blood pressure medications: Types, side effects, and risks [Internet]. 2018. Available from: https://www.medicalnewstoday.com/articles/323724

62. Larson EA, German DM, Shatzel J, DeLoughery TG. Anticoagulation in the cardiac patient: A concise review. Eur J Haematol. 2019;102(1):3–19.

63. Passacquale G, Sharma P, Perera D, Ferro A. Antiplatelet therapy in cardiovascular disease: Current status and future directions. Br J Clin Pharmacol. 2022;88(6):2686–99.

64. Usuda D, Higashikawa T, Hotchi Y, Usami K, Shimozawa S, Tokunaga S, et al. Angiotensin receptor blocker neprilysin inhibitors. World J Cardiol. 2021;13(8):325–39.

65. Elliott WJ, Ram CVS. Calcium channel blockers. J Clin Hypertens. 2011;13(9):687–9.

66. Pignone M, Phillips C, Mulrow C. Use of lipid lowering drugs for primary prevention of coronary heart disease: Meta-analysis of randomised trials. Vol. 321, British Medical Journal. 2000. p. 983–6.

67. Lloyd-Jones DM, Goff DC, Stone NJ. Guidelines for cardiovascular risk assessment and cholesterol treatment. Jama. 2014;311(21):2235.

68. Ganguly D, Chakraborty S, Balitanas M, Kim TH. Medical imaging: A review. Commun Comput Inf Sci. 2010;78 CCIS(February):504–16.

69. Zaidi H. Medical Imaging : Current status and future perspectives. Div Nucl Med Geneva Univ Hosp. 1997;1–21.

70. Bercovich E, Javitt MC. Medical Imaging: From Roentgen to the Digital Revolution, and Beyond. Rambam Maimonides Med J. 2018;9(4):e0034.

71. Hussain S, Mubeen I, Ullah N, Shah SSUD, Khan BA, Zahoor M, et al. Modern Diagnostic

Imaging Technique Applications and Risk Factors in the Medical Field: A Review. Biomed Res Int. 2022;2022.

72. Laal M. Innovation Process in Medical Imaging. Procedia - Soc Behav Sci [Internet]. 2013;81:60–4. Available from: http://dx.doi.org/10.1016/j.sbspro.2013.06.388

73. Waheed S, Tahir MJ, Ullah I, Alwalid O, Irshad SG, Asghar MS, et al. The impact of dependence on advanced imaging techniques on the current radiology practice. Ann Med Surg [Internet]. 2022;78(April):103708. Available from: https://doi.org/10.1016/j.amsu.2022.103708

74. Lammertsma AA. PET/SPECT: Functional imaging beyond flow. Vision Res. 2001;41(10–11):1277–81.

75. Mintzer RA, Aarsvold JN. Single-Photon Emission Computed Tomography. 2004;2004.

76. Sanders J. Emission Tomography. Lect Notes Comput Sci (including Subser Lect Notes Artif Intell Lect Notes Bioinformatics). 2018;11111 LNCS:207–36.

77. Dickson J, Ross J, Vöö S. Quantitative SPECT: the time is now. EJNMMI Phys. 2019;6(1):1–7.

78. Wagner RH. Practical Nuclear Medicine. Radiology. 1993;189(3):828–828.

79. Dorbala S, Ananthasubramaniam K, Armstrong IS, Chareonthaitawee P, DePuey EG, Einstein AJ, et al. Single Photon Emission Computed Tomography (SPECT) Myocardial Perfusion Imaging Guidelines: Instrumentation, Acquisition, Processing, and Interpretation. J Nucl Cardiol [Internet]. 2018;25(5):1784–846. Available from: https://doi.org/10.1007/s12350-018-1283-y

80. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, et al. Standardized myocardial sementation and nomenclature for tomographic imaging of the heart: A Statement for Healthcare Professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation. 2002;105(4):539–42.

81. Trinci M, Miele V. Jr Fs Jr. 2015;(I).

82. de Mendonça CR. Radionuclide therapy in nuclear medicine: applying Monte Carlo simulation to investigate Bremsstrahlung imaging with a gamma camera. 2013;(September):174. Available from: http://repositorio.ucp.pt/handle/10400.14/15659

83. Cao ZJ, Maunoury C, Chen CC, Holder LE. Comparison of continuous step-and-shoot versus step-and-shoot acquisition SPECT. J Nucl Med. 1996;37(12):2037–40.

84. Picone V, Makris N, Boutevin F, Roy S, Playe M, Soussan M. Clinical validation of time reduction strategy in continuous step-and-shoot mode during SPECT acquisition. EJNMMI Phys. 2021;8(1).

85. Radiology Key. The Gamma Camera: Basic Principles | Radiology Key [Internet]. 2016. Available from: https://radiologykey.com/the-gamma-camera-basic-principles/

86. Ilisie V, Moliner L, Oliver S, Sánchez F, González AJ, Seimetz M, et al. High resolution and sensitivity gamma camera with active septa. A first Monte Carlo study. Sci Rep. 2019;9(1):1–9.

87. IAEA. Nuclear Medicine Imaging Instrumentation. Rev Nucl Med Phys A Handb Teach Students. 2009;317–48.

88. Koppert WJC, Dietze MMA, Van Der Velden S, Steenbergen JHL, De Jong HWAM. A comparative study of NaI(Tl), CeBr3, and CZT for use in a real-time simultaneous nuclear and fluoroscopic dual-layer detector. Phys Med Biol. 2019;64(13).

89. Peterson TE, Furenlid LR. SPECT detectors: The Anger Camera and beyond. Phys Med Biol. 2011;56(17).

90. EANM. EANM_2017_TEchGuide_QualityControl. 2017;

91. Scintillators Material Group. Stanford: Advanced Optical Ceramics Laboratory [Internet]. 2013. Available from: https://web.stanford.edu/group/scintillators/scintillators.html

92. Elisa Crestoni M. Radiopharmaceuticals for Diagnosis and Therapy [Internet]. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Elsevier Inc.; 2018. 1–12 p. Available from: http://dx.doi.org/10.1016/B978-0-12-409547-2.14205-2

93. Oliveira R, Santos D, Ferreira D, Coelho P, Veiga F. Preparações radiofarmacêuticas e suas aplicações. Rev Bras Ciências Farm. 2006;42(2):151–65.

94. Hashimoto K, Nagai Y. Radionuclide Production. Compr Biomed Phys. 2014;8(3):219–27.

95. Kardjilov N, Woracek R, Manke I. Neutron Imaging [Internet]. Nanotechnologies and Nanomaterials for Diagnostic, Conservation and Restoration of Cultural Heritage. Elsevier Inc.; 2019. 47–59 p. Available from: http://dx.doi.org/10.1016/B978-0-12-813910-3.00003-3

96. Saha GB. Fundamentals of Nuclear Pharmacy, Fifth Edition. 2018.

97. An Evaluation of Radiation Exposure Guidance for Military Operations. An Evaluation of Radiation Exposure Guidance for Military Operations. 1997.

98. Albelda TM, Garcia-España E, Frias JC. Visualizing the atherosclerotic plaque: A chemical perspective. Chem Soc Rev. 2014;43(8):2858–76.

99. Robinson CN, Van Aswegen A, Julious SA, Nunan TO, Thomson WH, Tindale WB, et al. The relationship between administered radiopharmaceutical activity in myocardial perfusion scintigraphy and imaging outcome. Eur J Nucl Med Mol Imaging. 2008;35(2):329–35.

100. Strauss HW, Miller DD, Wittry MD, Cerqueira MD, Garcia E V., Iskandrian AS, et al. Procedure guideline for myocardial perfusion imaging 3.3. J Nucl Med Technol. 2008;36(3):155–61.

101. Lecchi M, Malaspina S, Scabbio C, Gaudieri V, Del Sole A. Myocardial perfusion scintigraphy dosimetry: optimal use of SPECT and SPECT/CT technologies in stress-first imaging protocol. Clin Transl Imaging. 2016;4(6):491–8.

102. Okada DR, Ghoshhajra BB, Blankstein R, Rocha-Filho JA, Shturman LD, Rogers IS, et al. Direct comparison of rest and adenosine stress myocardial perfusion CT with rest and stress SPECT. J Nucl Cardiol. 2010;17(1):27–37.

103. Gimelli A, Pugliese NR, Buechel RR, Coceani M, Clemente A, Kaufmann PA, et al. Myocardial perfusion scintigraphy for risk stratification of patients with coronary artery disease: The AMICO registry. Eur Heart J Cardiovasc Imaging. 2022;23(3):372–80.

104. DePuey EG, Mahmarian JJ, Miller TD, Einstein AJ, Hansen CL, Holly TA, et al. Patient-centered imaging. J Nucl Cardiol. 2012;19(2):185–215.

105. Cuocolo A. Challenges and opportunities of noninvasive cardiac imaging in obesity. J Nucl Cardiol. 2016;23(6):1233–4.

106. Anagnostopoulos C, Harbinson M, Kelion A, Kundley K, Loong CY, Notghi A, et al. Procedure guidelines for radionuclide myocardial perfusion imaging. Heart. 2004;90(SUPPL. 1):1–10.

107. Ritt P, Vija H, Hornegger J, Kuwert T. Absolute quantification in SPECT. Eur J Nucl Med Mol Imaging. 2011;38(SUPPL. 1):69–77.

108. Martin CJ, Sharp PF, Sutton DG. Measurement of image quality in diagnostic radiology. Appl Radiat Isot. 1999;50(1):21–38.

109. Mabotuwana T, Bhandarkar VS, Hall CS, Gunn ML. Detecting Technical Image Quality in Radiology Reports. AMIA . Annu Symp proceedings AMIA Symp. 2018;2018:780–8.

110. Williams MB, Krupinski EA, Strauss KJ, Breeden WK, Rzeszotarski MS, Applegate K, et al. Digital Radiography Image Quality: Image Acquisition. J Am Coll Radiol. 2007;4(6):371–88.

111. Zarb F, Rainford L, McEntee MF. Image quality assessment tools for optimization of CT images. Radiography [Internet]. 2010;16(2):147–53. Available from: http://dx.doi.org/10.1016/j.radi.2009.10.002

112. Mansson LG. Methods for the evaluation of image quality: A review. Radiat Prot Dosimetry. 2000;90(1–2):89–99.

113. Ma Y, Zhai Y, Yang C, Yang J, Wang R, Zhou J, et al. Variable rate ROI image compression optimized for visual quality. IEEE Comput Soc Conf Comput Vis Pattern Recognit Work. 2021;1936–40.

114. Boellaard R, Krak NC, Hoekstra OS, Lammertsma AA. Effects of noise, image resolution, and ROI definition on the accuracy of standard uptake values: A simulation study. J Nucl Med. 2004;45(9):1519–27.

115. Boellaard R, Krak NC, Hoekstra OS, Lammertsma A a, Jaskowiak CJ, Bianco J a, et al. Effects of noise, image resolution, and ROI definition on the accuracy of standard uptake values: a simulation study. J Nucl Med [Internet]. 2002;45(3):670–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15750154%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/15347719

116. Kurt A, Bor D, Tastan S, Donmez S, Haciosmanoğlu T. Accuracy of clinical protocols in SPECT. J Nucl Med Technol. 2012;40(4):259–64.

117. Kurkowska S, Birkenfeld B, Piwowarska-Bilska H. Physical quantities useful for quality control of quantitative SPECT/CT imaging. Nucl Med Rev. 2021;24(2):93–8.

118. Mamede FMB, Da Silva Gama ZA, Saturno-Hernández PJ. Improving the quality of radiological examinations: Effectiveness of an internal participatory approach. Int J Qual Heal Care. 2017;29(3):420–6.

119. Alsleem H, Davidson R. Quality parameters and assessment methods of digital radiography images. Radiographer. 2012;50(46).

120. Fafouti M, Paparrigopoulos T, Zervas Y, Rabavilas A, Malamos N, Liappas I, et al. Depression, anxiety and general psychopathology in breast cancer patients: A cross-sectional control study. In Vivo (Brooklyn). 2010;24(5):803–10.

121. Crocq MA. A history of anxiety: From Hippocrates to DSM. Dialogues Clin Neurosci. 2015;17(3):319–25.

122. Martin EI, Ressler KJ, Binder E, Nemeroff CB. The Neurobiology of Anxiety Disorders: Brain Imaging, Genetics, and Psychoneuroendocrinology. Psychiatr Clin North Am. 2009;32(3):549–75.

123. House A, Stark D. Anxiety in medical patients. Bmj. 2002;325(7357):207.

124. Cantekin I, Tan M. The influence of music therapy on perceived stressors and anxiety levels of hemodialysis patients. Ren Fail. 2013;35(1):105–9.

125. Patriquin MA, Mathew SJ. The Neurobiological Mechanisms of Generalized Anxiety Disorder and Chronic Stress. Chronic Stress. 2017;1.

126. Roxo MR, Franceschini PR, Zubaran C, Kleber FD, Sander JW. The limbic system conception and its historical evolution. ScientificWorldJournal. 2011;11:2427–40.

127. Darling D. Limbic System [Internet]. The World of David Darling. 2006. Available from: https://www.daviddarling.info/encyclopedia/L/limbic_system.html

128. Kötter R, Meyer N. The limbic system: a review of its empirical foundation. Behav Brain Res. 1992;52(2):105–27.

129. Kamali A, Milosavljevic S, Gandhi A, Lano KR, Shobeiri P, Sherbaf FG, et al. The Cortico-Limbo-Thalamo-Cortical Circuits: An Update to the Original Papez Circuit of the Human Limbic System. Brain Topogr [Internet]. 2023;36(3):371–89. Available from: https://doi.org/10.1007/s10548-023-00955-y

130. Parsapoor M. An introduction to brain emotional learning inspired models (BELiMs) with an example of BELiMs' applications. Artif Intell Rev [Internet]. 2019;52(1):409–39. Available from: https://doi.org/10.1007/s10462-018-9638-y

131. Buffum MD, Sasso C, Sands LP, Lanier E, Yellen M, Hayes A. A music intervention to reduce anxiety before vascular angiography procedures. J Vasc Nurs. 2006;24(3):68–73.

132. Domar AD, Eyvazzadeh A, Allen S, Roman K, Wolf R, Orav J, et al. Relaxation techniques for reducing pain and anxiety during screening mammography. Am J Roentgenol. 2005;184(2):445–7.

133. Kulkarni S, Johnson PCD, Kettles S, Kasthuri RS. Music during interventional radiological procedures, effect on sedation, pain and anxiety: A randomised controlled trial. Br J Radiol. 2012;85(1016):1059–63.

134. Lee WL, Sung HC, Liu SH, Chang SM. Meditative music listening to reduce state anxiety in patients during the uptake phase before positron emission tomography (PET) scans. Br J Radiol. 2017;90(1070).

135. Santangeli L, Lantheaume S, Eve K, Leclercq R. The luminous environment and patient anxiety in MRI service. Psychol Fr [Internet]. 2021;66(1):41–54. Available from: https://doi.org/10.1016/j.psfr.2020.05.001

136. Vanderboom TL, Arcari PM, Duffy ME, Somarouthu B, Rabinov JD, Yoo AJ, et al. Effects of a music intervention on patients undergoing cerebral angiography: A pilot study. J Neurointerv Surg. 2012;4(3):229–33.

137. Wen X, Shi J, Tan W, Jiang H, Wang D, Su J, et al. Effects of aromatherapy and music therapy on patients' anxiety during MRI examinations: a randomized controlled trial. Eur Radiol. 2022;

138. Spielberger CD, Gorsuch RL LR. STAI Manual. Consulting Psychologists Press. 1970.

139. Alacacioglu A, Binicier. O, Gungor O, Oztop I, Dirioz M, Yilmaz U. Quality of life, anxiety, and depression in Turkish colorectal cancer patients. Support Care Cancer. 2010;18(4):417–21.

140. Mystakidou K, Tsilika E, Parpa E, Gogou P, Theodorakis P, Vlahos L. Self-efficacy beliefs and levels of anxiety in advanced cancer patients. Eur J Cancer Care (Engl). 2010;19(2):205–11.

141. Sukegawa A, Miyagi E, Asai-Sato M, Saji H, Sugiura K, Matsumura T, et al. Anxiety and prevalence of psychiatric disorders among patients awaiting surgery for suspected ovarian cancer. J Obstet Gynaecol Res. 2008;34(4):543–51.

142. Kayikcioglu O, Bilgin S, Seymenoglu G, Deveci A. State and Trait Anxiety Scores of Patients Receiving Intravitreal Injections. Biomed Hub. 2017;2(2):1–5.

143. Greene J, Cohen D, Siskowski C, Toyinbo P. The Relationship Between Family Caregiving and the Mental Health of Emerging Young Adult Caregivers. J Behav Heal Serv Res. 2017;44(4):551–63.

144. Ugalde A, Krishnasamy M, Schofield P. The relationship between self-efficacy and anxiety and general distress in caregivers of people with advanced cancer. J Palliat Med. 2014;17(8):939–41.

145. Delgado DA, Lambert BS, Boutris N, McCulloch PC, Robbins AB, Moreno MR, et al. Validation of Digital Visual Analog Scale Pain Scoring With a Traditional Paper-based Visual Analog Scale in Adults. J Am Acad Orthop Surg Glob Res Rev. 2018;2(3).

146. Klimek L, Bergmann KC, Biedermann T, Bousquet J, Hellings P, Jung K, et al. Visual

analogue scales (VAS) - Measuring instruments for the documentation of symptoms and therapy monitoring in case of allergic rhinitis in everyday health care. Allergo J. 2017;26(1):36–47.

147. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, Loge JH, et al. Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for assessment of pain intensity in adults: A systematic literature review. J Pain Symptom Manage [Internet]. 2011;41(6):1073–93. Available from: http://dx.doi.org/10.1016/j.jpainsymman.2010.08.016

148. Yeung AWK, Wong NSM. The historical roots of visual analog scale in psychology as revealed by reference publication year spectroscopy. Front Hum Neurosci. 2019;13(March):1–5.

149. Ducoulombier V, Chiquet R, Graf S, Leroy B, Bouquet G, Verdun S, et al. Usefulness of a Visual Analog Scale for Measuring Anxiety in Hospitalized Patients Experiencing Pain: A Multicenter Cross-Sectional Study. Pain Manag Nurs. 2020;21(6):572–8.

150. Hernández-Palazón J, Fuentes-García D, Falcón-Araña L, Rodríguez-Ribó A, García-Palenciano C, Roca-Calvo MJ. Visual analogue scale for anxiety and Amsterdam preoperative anxiety scale provide a simple and reliable measurement of preoperative anxiety in patients undergoing cardiac surgery. Int Cardiovasc Res J. 2015;9(1):1–6.

151. Facco E, Zanette G, Favero L, Bacci C, Sivolella S, Cavallin F, et al. Toward the validation of visual analogue scale for anxiety. Anesth Prog. 2011;58(1):8–13.

152. Cao X, Yumul R, Lazo OLE, Friedman J, Durra O, Zhang X, et al. A novel visual facial anxiety scale for assessing preoperative anxiety. PLoS One. 2017;12(2):1–7.

153. Łoś K, Waszkiewicz N. Biological markers in anxiety disorders. J Clin Med. 2021;10(8).

154. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009;34(2):163–71.

155. Vreeburg SA, Zitman FG, Van Pelt J, Derijk RH, Verhagen JCM, Van Dyck R, et al. Salivary cortisol levels in persons with and without different anxiety disorders. Psychosom Med. 2010;72(4):340–7.

156. Dziurkowska E, Wesolowski M. Cortisol as a biomarker of mental disorder severity. J Clin Med. 2021;10(21).

157. Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity. Clin Endocrinol (Oxf). 2005;63(3):336–41.

158. Chojnowska S, Ptaszy´nska I, Ptaszy´nska-Sarosiek P, K¸ Epka A, Kna´s MK, Waszkiewicz N. Clinical Medicine Salivary Biomarkers of Stress, Anxiety and Depression. J Clin Med. 2021;10.

159. Schwartz EB, Granger DA, Susman EJ, Gunnar MR, Laird B. Assessing Salivary Cortisol in Studies of Child Development. Child Dev. 1998;69(6):1503–13.

160. Clow A, Hucklebridge F, Thorn L. The cortisol awakening response in context [Internet]. Vol. 93, International Review of Neurobiology. Elsevier Inc.; 2010. 153–175 p. Available from: http://dx.doi.org/10.1016/S0074-7742(10)93007-9

161. Gröschl M. Current status of salivary hormone analysis. Clin Chem. 2008;54(11):1759–69.

162. Schoofs D, Hartmann R, Wolf OT. Neuroendocrine stress responses to an oral academic examination: No strong influence of sex, repeated participation and personality traits. Stress. 2008;11(1):52–61.

163. Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol Bull. 2007;133(1):25–45.

164. Invitrogen. Cortisol Competitive ELISA kit. 2018;4–7. Available from: https://www.thermofisher.com/elisa/product/Cortisol-Competitive-Human-ELISA-Kit/EIAHCOR

165. Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides [Internet]. 2015;72:4–15. Available from: http://dx.doi.org/10.1016/j.peptides.2015.04.012

166. Shah K, Maghsoudlou P. Enzyme-linked immunosorbent assay (ELISA): The basics. Br J Hosp Med. 2016;77(7):C98–101.

167. Mariani M, Luzzi E, Proietti D, Mancianti S, Casini D, Costantino P, et al. A competitive enzyme-linked immunosorbent assay for measuring the levels of serum antibody to Haemophilus influenzae type b. Clin Diagn Lab Immunol. 1998;5(5):667–74.

168. Katzman MA, Bleau P, Blier P, Chokka P, Kjernisted K, Van Ameringen M, et al. Canadian clinical practice guidelines for the management of anxiety, posttraumatic stress and obsessive-compulsive disorders. BMC Psychiatry. 2014;14(SUPPL.1):1–83.

169. Bradley YC, Barlow P, Osborne DR. Reduction of patient anxiety in PET/CT imaging by improving communication between patient and technologist. J Nucl Med Technol. 2014;42(3):211–7.

170. Kim SK, Jeong HC. Effects of patient-selected music listening on the pain and anxiety of patients undergoing hemodialysis: A randomized controlled trial. Healthc. 2021;9(11).

171. Hewis J. Music and Music Therapy in the Medical Radiation Sciences. J Med Imaging Radiat Sci [Internet]. 2018;49(4):360–4. Available from: https://doi.org/10.1016/j.jmir.2018.09.009

172. Bradt J, Potvin N, Kesslick A, Shim M, Radl D, Schriver E, et al. The impact of music therapy versus music medicine on psychological outcomes and pain in cancer patients: a mixed methods study. Support Care Cancer. 2015;23(5):1261–71.

173. Gold C, Erkkilä J, Bonde LO, Trondalen G, Maratos A, Crawford MJ. Music therapy or music medicine? Psychother Psychosom. 2011;80(5):304.

174. Robb SL, Hanson-Abromeit D, May L, Hernandez-Ruiz E, Allison M, Beloat A, et al. Reporting quality of music intervention research in healthcare: A systematic review. Complement Ther Med [Internet]. 2018;38(December 2017):24–41. Available from: https://doi.org/10.1016/j.ctim.2018.02.008

175. Yinger OS wedber., Gooding LF. A systematic review of music-based interventions for procedural support. J Music Ther. 2015;52(1):1–77.

176. Bradt J, Dileo C, Shim M. Music interventions for preoperative anxiety. Vol. 2013, Cochrane Database of Systematic Reviews. 2013.

177. Los UMDECDE. American Music Therapy Association. 2014;

178. WFMT. World Federation of Music Therapy [Internet]. Announcing WFMT´s New Definition of music Therapy. 2011. Available from: https://wfmt.info/%0Ahttps://www.wfmt.info/2011/05/01/announcing-wfmts-new-definition-of-music-therapy/#:~:text=“Music therapy is the professional,%2C emotional%2C intellectual%2C and spiritual

179. Moore M. Music Therapy in Adult Medical Settings : Recommendations and Advocacy for Sustainable Programs Honors Thesis. 2022;

180. Van Der Heijden MJE, Araghi SO, Van Dijk M, Jeekel J, Hunink MGM. The effects of perioperative music interventions in pediatric surgery: A systematic review and meta-analysis of randomized controlled trials. PLoS One. 2015;10(8):1–11.

181. Edwards J. A Reflection on the Music Therapist’s Role in Developing a Program in a Children’s Hospital. Music Ther Perspect. 2005;23(1):36–44.

182. Guétin S, Brun L, Deniaud M, Clerc JM, Thayer JF, Koenig J. Smartphone-based Music Listening to Reduce Pain and Anxiety Before Coronarography: A Focus on Sex Differences. Altern Ther Health Med. 2016;22(4):60–3.

183. Tan YZ, Ozdemir S, Temiz A, Celik F. The effect of relaxing music on heart rate and heart rate variability during ECG GATED-myocardial perfusion scintigraphy. Complement Ther Clin Pract [Internet]. 2015;21(2):137–40. Available from: http://dx.doi.org/10.1016/j.ctcp.2014.12.003

184. Stanley E, Cradock A, Bisset J, McEntee C, O'connell MJ. Impact of sensory design interventions on image quality, patient anxiety and overall patient experience at MRI. Br J Radiol. 2016;89(1067):1–6.

185. Weeks BP, Nilsson U. Music interventions in patients during coronary angiographic procedures: A randomized controlled study of the effect on patients' anxiety and well-being. Eur J Cardiovasc Nurs [Internet]. 2011;10(2):88–93. Available from: http://dx.doi.org/10.1016/j.ejcnurse.2010.07.002

186. Moradipanah F, Mohammadi E, Mohammadil AZ. Effect of music on anxiety, stress, and depression levels in patients undergoing coronary angiography. East Mediterr Heal J. 2009;15(3):639–47.

187. Kühlmann AYR, de Rooij A, Kroese LF, van Dijk M, Hunink MGM, Jeekel J. Meta-analysis evaluating music interventions for anxiety and pain in surgery. Br J Surg. 2018;105(7):773–83.

188. Balasopoulou A, Kokkinos P, Pagoulatos D, Plotas P, Makri OE, Georgakopoulos CD, et al. Symposium Recent advances and challenges in the management of retinoblastoma Globe - saving Treatments. BMC Ophthalmol. 2017;17(1):1.

189. Klassen JA, Liang Y, Tjosvold L, Klassen TP, Hartling L. Music for Pain and Anxiety in Children Undergoing Medical Procedures: A Systematic Review of Randomized Controlled Trials. Ambul Pediatr. 2008;8(2):117–28.

190. Lin CJ, Chang YC, Chang YH, Hsiao YH, Lin HH, Liu SJ, et al. Music interventions for anxiety in pregnant women: A systematic review and meta-analysis of randomized controlled trials. J Clin Med. 2019;8(11):1–12.

191. Su SF, Yeh WT. Music Interventions in Percutaneous Coronary Procedures: A Meta-Analysis. Clin Nurs Res. 2021;30(2):135–45.

192. Hole J, Hirsch M, Ball E, Meads C. Music as an aid for postoperative recovery in adults: A systematic review and meta-analysis. Lancet [Internet]. 2015;386(10004):1659–71. Available from: http://dx.doi.org/10.1016/S0140-6736(15)60169-6

193. Nguyen KT, Xiao J, Chan DNS, Zhang M, Chan CWH. Effects of music intervention on anxiety, depression, and quality of life of cancer patients receiving chemotherapy: a systematic review and meta-analysis. Support Care Cancer [Internet]. 2022;30(7):5615–26. Available from: https://doi.org/10.1007/s00520-022-06881-2

194. Erkkilä J, Punkanen M, Fachner J, Ala-Ruona E, Pöntiö I, Tervaniemi M, et al. Individual music therapy for depression: Randomised controlled trial. Br J Psychiatry. 2011;199(2):132–9.

195. Zengin S, Kabul S, Al B, Sarcan E, Doğan M, Yildirim C. Effects of music therapy on pain and anxiety in patients undergoing port catheter placement procedure. Complement Ther Med. 2013;21(6):689–96.

196. Huang ST, Good M, Zauszniewski JA. The effectiveness of music in relieving pain in cancer patients: A randomized controlled trial. Int J Nurs Stud [Internet]. 2010;47(11):1354–62. Available from: http://dx.doi.org/10.1016/j.ijnurstu.2010.03.008

197. Çift A, Benlioglu C. Effect of different musical types on patient's relaxation, anxiety and pain perception during shock wave lithotripsy: A randomized controlled study. Urol J. 2020;17(1):19–23.

198. Hanedan Uslu G. Influence of music therapy on the state of anxiety during radiotherapy. Turk Onkol Derg. 2017;32(4):141–7.

199. Fleckenstein FN, Böhm AK, Collettini F, Frisch A, Lüdemann WM, Can E, et al. A prospective randomized controlled trial assessing the effect of music on patients' anxiety in venous catheter placement procedures. Sci Rep [Internet]. 2022;12(1):1–8. Available from: https://doi.org/10.1038/s41598-022-10862-0

200. Danhauer SC, Vishnevsky T, Campbell CR, McCoy TP, Tooze JA, Kanipe KN, et al. Music for patients with hematological malignancies undergoing bone marrow biopsy: A randomized controlled study of anxiety, perceived pain, and patient satisfaction. J Soc Integr Oncol. 2010;8(4):140–7.

201. Ko SY, Leung DYP, Wong EML. Effects of easy listening music intervention on satisfaction, anxiety, and pain in patients undergoing colonoscopy: A pilot randomized controlled trial. Clin Interv Aging. 2019;14:977–86.

202. Mazhari-Jensen DS, Jacobsen SL, Jespersen KV. Inpatient stroke survivors with low gait functioning benefit from music interventions during cardiorespiratory exercise: A randomized cross-over trial. Nord J Music Ther [Internet]. 2023;00(00):1–20. Available from: https://doi.org/10.1080/08098131.2023.2190403

203. Bernardi NF, Codrons E, di Leo R, Vandoni M, Cavallaro F, Vita G, et al. Increase in synchronization of autonomic rhythms between individuals when listening to music. Front Physiol. 2017;8(OCT):1–10.

204. Leslie G, Ghandeharioun A, Zhou D, Picard RW. Engineering Music to Slow Breathing and Invite Relaxed Physiology. 2019 8th Int Conf Affect Comput Intell Interact ACII 2019. 2019;276–82.

205. Bernardi L, Porta C, Casucci G, Balsamo R, Bernardi NF, Fogari R, et al. Dynamic interactions between musical, cardiovascular, and cerebral rhythms in humans. Circulation. 2009;119(25):3171–80.

206. Sebastiani L, Mastorci F, Magrini M, Paradisi P, Pingitore A. Synchronization between music dynamics and heart rhythm is modulated by the musician's emotional involvement: A single case study. Front Psychol. 2022;13(September):1–8.

207. Fukumoto M, Nomura S, Sawai M, Imai J, Nagashima T. Investigation of synchronization between musical beat and heartbeat with cardio-music synchrogram. Nonlinear Theory Its Appl IEICE. 2010;1(1):146–52.

208. Selle EW, Silverman MJ. Cardiovascular patients' perceptions of music therapy in the form of patient-preferred live music: Exploring service user experiences. Nord J Music Ther [Internet]. 2020;29(1):57–74. Available from: https://doi.org/10.1080/08098131.2019.1663245

209. El Boghdady M, Ewalds-Kvist BM. The influence of music on the surgical task performance: A systematic review. Int J Surg [Internet]. 2020;73(October 2019):101–12. Available from: https://doi.org/10.1016/j.ijsu.2019.11.012

210. Katz JD. Noise in the operating room. Anesthesiology. 2014;121(4):894–8.

211. Ghetti CM. Effect of music therapy with emotional- Approach coping on preprocedural anxiety in cardiac catheterization: A randomized controlled trial. J Music Ther. 2013;50(2):93–122.

212. Batista L de C, Melo MN, Cruz D de ALM da, Gengo e Silva Butcher R de C. Characteristics of music intervention to reduce anxiety in patients undergoing cardiac catheterization: scoping review. Heliyon. 2022;8(11).

213. Walworth DD. Effect of live music therapy for patients undergoing magnetic resonance imaging. J Music Ther. 2010;47(4):335–50.

214. Ma D, Pierre EY, Jiang Y, Schluchter MD, Setsompop K, Gulani V, et al. Music-based

magnetic resonance fingerprinting to improve patient comfort during MRI examinations. Magn Reson Med. 2016;75(6):2303–14.

215. Invitrogen. LaboShop _ Products _ Invitrogen Cortisol Competitive Human ELISA Kit, 96 Tests. 2021.

216. Chemistry S and share. Cortisol | C21H30O5 | [Internet]. 2017. Available from: http://www.chemspider.com/Chemical-Structure.5551.html

217. GE HealthCare _ GE HealthCare (United States).

218. Google Play. Decibel X - Sonómetro Pro. 2023.

219. Hamacher D, Schley F, Hollander K, Zech A. Effects of manipulated auditory information on local dynamic gait stability. Hum Mov Sci [Internet]. 2018;58(February):219–23. Available from: https://doi.org/10.1016/j.humov.2018.02.010

220. Gordon RT, Vining WD. Active noise control: A review of the field. Am Ind Hyg Assoc J. 1992;53(11):721–5.

221. Akhtar S, Weigle CGM, Cheng EY, Toohill R, Berens RJ. Use of active noise cancellation devices in caregivers in the intensive care unit. Crit Care Med. 2000;28(4):1157–60.

222. Knight W, Rickard N. Relaxing music prevents stress-induced increases in subjective anxiety, …. J Music Ther [Internet]. 2001;(4):254–72. Available from: http://www.chinamusictherapy.org/file/doc/Relaxing Music Prevents Stress-Induced Increases in Subjective Anxiety, Systolic Blood Pressure, and Heart Rate in Healthy.pdf

223. Harmat L, Takács J, Bódizs R. Music improves sleep quality in students. J Adv Nurs. 2008;62(3):327–35.

224. Trappe HJ. The effects of music on the cardiovascular system and cardiovascular health. Heart. 2010;96(23):1868–71.

225. Spotify. Spotify Premium [Internet]. Spotify. 2016. Available from: https://www.spotify.com/ch-de/

226. Koninklijke P. The cost of fear and anxiety in radiology. 2020;

227. Koutsimani P, Montgomery A, Georganta K. The relationship between burnout, depression, and anxiety: A systematic review and meta-analysis. Front Psychol. 2019;10(MAR):1–19.

228. Bahrami F, Yousefi N. Females are more anxious than males: A metacognitive perspective. Iran J Psychiatry Behav Sci. 2011;5(2):83–90.

229. Hallers-Haalboom ET, Maas J, Kunst LE, Bekker MHJ. The role of sex and gender in anxiety disorders: Being scared “like a girl”? [Internet]. 1st ed. Vol. 175, Handbook of Clinical Neurology. Elsevier B.V.; 2020. 359–368 p. Available from: http://dx.doi.org/10.1016/B978-0-444-64123-6.00024-2

230. Eberhart L, Aust H, Schuster M, Sturm T, Gehling M, Euteneuer F, et al. Preoperative anxiety in adults - A cross-sectional study on specific fears and risk factors. BMC Psychiatry. 2020;20(1):1–14.

231. Legg AM, Andrews SE, Huynh H, Ghane A, Tabuenca A, Sweeny K. Patients’ anxiety and hope: Predictors and adherence intentions in an acute care context. Heal Expect. 2015;18(6):3034–43.

232. Folk J, Folk M. Confusion Anxiety Disorder Symptoms - AnxietyCentre.com [Internet]. Anxietycentre. 2021. p. 1. Available from: https://www.anxietycentre.com/anxiety-disorders/symptoms/confusion-anxiety/

233. Labaste F, Ferré F, Combelles H, Rey V, Foissac JC, Senechal A, et al. Validation of a visual analogue scale for the evaluation of the postoperative anxiety: A prospective observational study. Nurs Open. 2019;6(4):1323–30.

234. Gustafson LW, Gabel P, Hammer A, Lauridsen HH, Petersen LK, Andersen B, et al. Validity and reliability of State-Trait Anxiety Inventory in Danish women aged 45 years and older with abnormal cervical screening results. BMC Med Res Methodol. 2020;20(1):1–9.

235. Ivanovs R, Kivite A, Ziedonis D, Mintale I, Vrublevska J, Rancans E. Association of depression and anxiety with the 10-year risk of cardiovascular mortality in a primary care population of Latvia using the SCORE system. Front Psychiatry. 2018;9:1–14.

236. Berman JJ. Indispensable Tips for Fast and Simple Big Data Analysis. Princ Pract Big Data. 2018;231–57.

237. Koskinen MK, Hovatta I. Genetic insights into the neurobiology of anxiety. Trends Neurosci [Internet]. 2023;46(4):318–31. Available from: https://doi.org/10.1016/j.tins.2023.01.007

238. Agilent Technologies. Sample Preparation Fundametal for Chormatography. Agilent Technologies, Mississauga, Canada. 2013. 364 p.

239. Nybo M, Cadamuro J, Cornes MP, Gómez Rioja R, Grankvist K. Sample transportation - An overview. Diagnosis. 2019;6(1):39–43.

240. Murphy JM, Browne RW, Hill L, Bolelli GF, Abagnato C, Berrino F, et al. Effects of transportation and delay in processing on the stability of nutritional and metabolic biomarkers. Nutr Cancer. 2000;37(2):155–60.

241. Malentacchi F, Pizzamiglio S, Wyrich R, Verderio P, Ciniselli C, Pazzagli M, et al. Effects of Transport and Storage Conditions on Gene Expression in Blood Samples. Biopreserv Biobank. 2016;14(2):122–8.

242. Wetsch WA, Pircher I, Lederer W, Kinzl JF, Traweger C, Heinz-Erian P, et al. Preoperative stress and anxiety in day-care patients and inpatients undergoing fast-track surgery. Br J Anaesth [Internet]. 2009;103(2):199–205. Available from: http://dx.doi.org/10.1093/bja/aep136

243. Andrea M, Brizard B, Dujardin PA, Réméniéras J-P, Frédéric P, Gissot V, et al. When Classical Music Relaxes the Brain: an experimental study using Ultrasound Brain Tissue Pulsatility Imaging. Int J Psychophysiol. 2020;1–22.

244. Thaut MH, McIntosh GC, Hoemberg V. Neurobiological foundations of neurologic music therapy: rhythmic entrainment and the motor system. Front Psychol. 2015;5(February):1–6.

245. Golden TL, Springs S, Kimmel HJ, Gupta S, Tiedemann A, Sandu CC, et al. The Use of Music in the Treatment and Management of Serious Mental Illness: A Global Scoping Review of the Literature. Front Psychol. 2021;12(March):1–19.

# 14 Communications and Publications

## 14.1 Oral communications

National conferences and seminars

- Lina Vieira, Catarina Carvalho, Marina Almeida-Silva, Jaime Reis, Ana Grilo, Ana Pires, Luísa Veiga, Catarina Ginete, Edgar Pereira, Luís Oliveira, Ana Abreu, Elisabete Carolino. Myocardial Perfusion Scintigraphy: Impact of Anxiety on Image Quality. Bootcamp do H&TRC, December 18th, 2022.

- Lina Vieira, Catarina Carvalho, Marina Almeida-Silva, Jaime Reis, Ana Grilo, Ana Pires, Luísa Veiga, Catarina Ginete, Edgar Pereira, Luís Oliveira, Ana Abreu, Elisabete Carolino. Avaliação da ansiedade em pacientes submetidos a Cintigrafia de Perfusão do Miocárdio. V Congresso Internacional de Imagem Médica e Radioterapia e VI Congresso de Imagem Médica e Radioterapia da ESALD, May 26-27th, 2023.

- Lina Vieira, Catarina Carvalho, Marina Almeida-Silva, Jaime Reis, Ana Grilo, Ana Pires, Luísa Veiga, Catarina Ginete, Edgar Pereira, Luís Oliveira, Ana Abreu, Elizabete Carolino. Impact of anxiety on myocardial perfusion scintigraphy image quality. Bootcamp from H&TRC, July 10th , 2023.

- Catarina Carvalho, Lina Vieira, Marina Almeida-Silva, Jaime Reis, Ana Grilo, Ana Pires, Luísa Veiga, Catarina Ginete, Edgar Pereira, Luís Oliveira, Ana Abreu, Elizabete Carolino. Impact of anxiety on myocardial perfusion scintigraphy image quality. XX CNATARP, November 4th, 2023. Accepted

Poster communications

- Catarina Carvalho, Marina Almeida-Silva, Jaime Reis, Ana Grilo, Ana Pires, Edgar Pereira, Lina Vieira. Reducing mental health risks during diagnostic imaging: a systematic review on anxiety reduction strategies through music. 4th Annual meeting of the Society for risk Analysis- Europe Iberian Chapter. Lisboa, April 13th , 2023.

International conferences and seminars

- Catarina Carvalho, Marina Almeida-Silva, Jaime Reis, Ana Grilo, Ana Pires, Luísa Veiga, Catarina Ginete, Edgar Pereira, Luís Oliveira, Ana Abreu, Elizabete Carolino, Luísa Vieira. Myocardial Perfusion Scintigraphy: Impact of Patient Anxiety on Image Quality. ICOMET 23, Dessel, Belgica, August 31st , 2023.

## 14.2 Publications

- L. Vieira, C. Carvalho, A. Grilo, J. Reis, A. Pires, E. Pereira, M. Almeida-Silva. Effects of a music-based intervention on psychophysiological outcomes of patients undergoing imaging procedures: a systematic review. Accepted and analysed by reviewers, October 4th, 2023.

# APPENDICES

APPENDIX 1: Informed consent for participants.

**CONSENTIMENTO INFORMADO, LIVRE E ESCLARECIDO PARA PARTICIPAÇÃO EM INVESTIGAÇÃO**

**de acordo com a Declaração de Helsínquia[1] e a Convenção de Oviedo[2]**

*Por favor, leia com atenção a seguinte informação. Se achar que algo está incorreto ou que não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.*

**"Título do estudo:** "Cintigrafia de Perfusão do Miocárdio: Impacto da Ansiedade na Qualidade de Imagem"

**Enquadramento**: Este projeto de investigação do centro de investigação H&TRC está a ser desenvolvido com a colaboração da clínica *NuclearMed,* em Almada, especificamente em pacientes que tenham indicação clínica para fazer o exame de cintigrafia de perfusão do miocárdio, neste serviço de Medicina Nuclear.
Salienta-se ainda que, o projeto é coordenado pela Doutora Lina Vieira, da Escola Superior de Tecnologia de Saúde de Lisboa – Instituto Politécnico de Lisboa.

**Explicação do estudo**: O estudo tem como principal objetivo avaliar o impacto da ansiedade em pacientes que realizam exames de cintigrafia de perfusão do miocárdio e consequentemente na qualidade das imagens médicas obtidas. Pretende-se com este estudo melhorar o conforto e segurança dos doentes que realizam estes exames na clínica *NuclearMed* e consequentemente a qualidade de imagem e do resultado final.

**Finalidade do tratamento de dados pessoais**: Serão recolhidos os seguintes dados pessoais: género, idade, habilitações académicas, situação laboral e indicação clínica. Estes dados destinam-se à caraterização geral da amostra do estudo. Será ainda solicitado o endereço de correio eletrónico, morada e contacto telefónico para que, seja enviada as salivette para recolha da saliva, quatro dias anteriores ao exame, e seja possível relembrar para não se esquecer de fazer a recolha da saliva para a salivette. Para além disso, no dia do exame, serão recolhidas mais três amostras de saliva e será registado o valor da tensão arterial, número de batimentos por minuto, frequência respiratória e nível de oxigénio medido no sangue, assim como informações relacionadas com o nível de ansiedade recolhida através dos questionários STAI.
Os dados recolhidos são para uso exclusivo do presente estudo, não existindo quaisquer interesses financeiros a motivar o estudo, nem transferência dos seus dados para outros investigadores que não integrem o projeto.

**Confidencialidade e conservação dos dados**: A confidencialidade dos dados serão garantidos. A identificação far-se-á por um código e, após análise de toda a informação recolhida, e divulgação pela comunidade científica dos resultados gerais, os dados serão armazenados durante 5 anos num PC da ESTeSL, à guarda do investigador principal Prof. Lina Vieira.

**Encarregado da Proteção de Dados**: Nuno Pires Encarregado da Proteção Dados / Data Protection Officer, Telf. + 351 21 046 47 00 | + 351 21 046 47 08, Email. npires@net.ipl.pt

[1] http://portal.arsnorte.min-saude.pt/portal/page/portal/ARSNorte/Comiss%C3%A3o%20de%20%C3%89tica/Ficheiros/Declaracao_Helsinquia_2008.pdf

[2] http://dre.pt/pdf1sdip/2001/01/002A00/00140036.pdf

APPENDIX 2: Sociodemographic questionnaire.

CPMeAQI_Qpré_____

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA

**CPM_ PRE-EXAM FORM**
(Grilo, et al., 2017, adapted)

1. **Gender:**
   ☐ Female ☐ Male
2. **Age:** _____
3. **Civil Status** _____

4. **Academic degree:**
   ☐ Without habilitations
   ☐ Até 9.º ano
   ☐ High School
   ☐ Bachelor's degree
   ☐ Master degree/PhD

5. **Occupation:**

**5.1. Professional situation at present:**
   ☐ Working
   ☐ Medical leave
   ☐ Retired
   ☐ Part-time worker
   ☐ Unemployed

**5.2.** Do you work or have you worked in the healthcare field? ☐ Yes ☐ No

**6.** Do you know why you are taking this exam today?

☐Yes ☐No (If the answer is "No", go to question 7.)

**6.1.** If yes, indicate the reason _____

**7.** Have you previously performed other imaging tests, such as Magnetic Resonance Imaging, CT scan or other scintigraphy?

☐Yes ☐No

**7.1.** How do you rate the experience of these exams?

| Very Hard | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Very Easy |
|---|---|---|---|---|---|---|---|---|---|---|---|

APPENDIX 3: STAI-S questionnaire.

CPMeAQI_STAI-S pre

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA

**STAI-S**

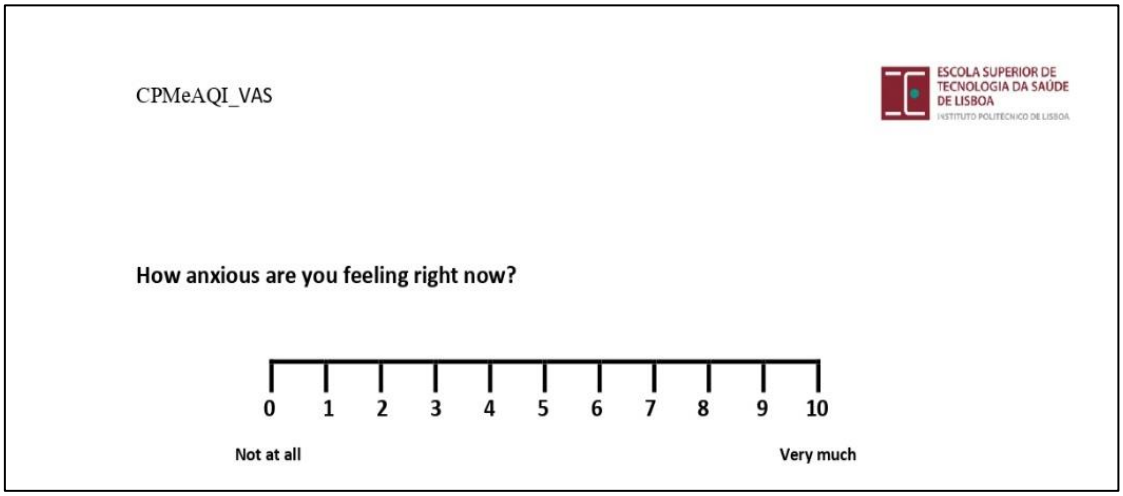
(Spielberger, Gorsuch & Lushene, 1983; versão portuguesa Silva, 2003)

| | Not at all | A little | Somewhat | Very much so |
|---|---|---|---|---|
| 1-I feel calm | 0 | 1 | 2 | 3 |
| 2- I feel secure | 0 | 1 | 2 | 3 |
| 3- I feel tense | 0 | 1 | 2 | 3 |
| 4- I feel strained | 0 | 1 | 2 | 3 |
| 5- I feel at ease | 0 | 1 | 2 | 3 |
| 6- I feel up set | 0 | 1 | 2 | 3 |
| 7- I am presently worrying over possible misfortunes | 0 | 1 | 2 | 3 |
| 8- I feel satisfied | 0 | 1 | 2 | 3 |
| 9- I feel frightened | 0 | 1 | 2 | 3 |
| 10- I feel uncomfortable | 0 | 1 | 2 | 3 |
| 11- I feel self-confident | 0 | 1 | 2 | 3 |
| 12-I feel nervous | 0 | 1 | 2 | 3 |
| 13- I feel jittery | 0 | 1 | 2 | 3 |
| 14- I feel indecisive | 0 | 1 | 2 | 3 |
| 15- I am relaxed | 0 | 1 | 2 | 3 |
| 16- I feel content | 0 | 1 | 2 | 3 |
| 17- I am worried | 0 | 1 | 2 | 3 |
| 18- I feel confused | 0 | 1 | 2 | 3 |
| 19- I feel stabled | 0 | 1 | 2 | 3 |
| 20- I feel good | 0 | 1 | 2 | 3 |

**Please verify if you answer all the questions.**

**We appreciate the collaboration!**

APPENDIX 4: VAS questionnaire.



APPENDIX 5: Sociodemographic data for Cross-sectional study.

| ID | Age | Gender | Prior Exams | Difficulty | Medical work | Types of CVRFs | | | | | Medication | Terapeutic group | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 02 | 75 | M | N | ----- | N | | | | | | No information | | | | |
| 03 | 68 | F | S | 1 | N | | | | | | No information | | | | |
| 04 | 73 | F | S | 3 | N | HBP | Dyslipidemia | Tabagism | | | 1 | ACE | | | |
| 05 | 72 | F | N | ----- | N | HBP | Dyslipidemia | | Obesity | | 3 | ACE | BB | ARBs | |
| 06 | 52 | F | N | ----- | N | HBP | Dyslipidemia | | | | 2 | ACE | BB | | |
| 07 | 62 | M | N | ----- | N | | | | | | No information | | | | |
| 08 | 70 | F | N | ----- | N | HBP | | | | | 2 | ACE | BB | | |
| 09 | 60 | F | S | 8 | S | | | | | | No information | | | | |
| 10 | 47 | M | S | 3 | N | | | Tabagism | | | No information | | | | |
| 11 | 67 | M | N | ----- | N | | | | | | No information | | | | |
| 01 | 76 | M | N | ----- | N | | | | | | No information | | | | |
| 12 | 56 | F | N | ----- | N | HBP | | | | | No information | | | | |
| 13 | 64 | M | S | 3 | N | HBP | Dyslipidemia | | | T2DM | 2 | | BB | ARBs | |
| 14 | 74 | M | S | 10 | N | HBP | Dyslipidemia | Tabagism | | | 3 | ACE | BB | | CCB |
| 15 | 80 | M | S | 3 | N | HBP | Dyslipidemia | Tabagism | | | 1 | ACE | | | |
| 17 | 66 | F | N | ----- | N | | Dyslipidemia | | | | 1 | ACE | | | |
| 18 | 77 | F | N | ----- | N | HBP | Dyslipidemia | | | | 1 | ACE | | | |
| 19 | 39 | F | S | 10 | N | HBP | | Tabagism | | | 1 | | BB | | |
| 20 | 43 | F | N | ----- | N | HBP | | | | | 1 | | BB | | |
| 21 | 83 | M | N | ----- | N | HBP | Dyslipidemia | Tabagism | | | 1 | | BB | | |
| 22 | 76 | F | S | 6 | N | | Dyslipidemia | | | | 0 | | | | |
| 23 | 68 | M | S | 10 | N | HBP | Dyslipidemia | Tabagism | | | 2 | ACE | BB | | |
| 26 | 70 | F | S | 4 | N | HBP | | | | | 2 | | BB | | CCB |
| 27 | 69 | M | S | 8 | N | HBP | Dyslipidemia | | | T2DM | 2 | ACE | | | CCB |
| 28 | 81 | M | S | 10 | N | | | | | | 0 | | | | |
| 29 | 59 | F | S | 8 | N | | | | Obesity | | 0 | | | | |
| 30 | 38 | F | N | ----- | N | | | | | | No information | | | | |
| 31 | 81 | F | S | 10 | N | HBP | | | Obesity | | 3 | | BB | ARBs | CCB |
| 16 | 76 | F | N | ----- | N | HBP | Dyslipidemia | | | | 2 | ACE | | | CCB |
| 24 | 74 | M | N | ----- | N | | Dyslipidemia | Tabagism | | | 0 | | | | |
| 25 | 57 | M | S | 8 | N | | Dyslipidemia | Tabagism | | | 0 | | | | |
| 34 | 76 | F | N | ----- | N | HBP | | | | | 1 | ACE | | | |
| 35 | 77 | F | N | ----- | N | HBP | | | | T2DM | 2 | | | ARBs | CCB |
| 36 | 65 | F | S | 10 | N | HBP | Dyslipidemia | | | | 1 | | | ARBs | |
| 37 | 61 | M | N | ----- | N | | | | | | No information | | | | |
| 38 | 77 | M | S | 8 | N | | | | | | No information | | | | |
| 40 | 57 | M | N | ----- | N | HBP | Dyslipidemia | Tabagism | Obesity | T2DM | 1 | ACE | | | |
| 32 | 58 | M | S | 8 | N | | | | | | No information | | | | |
| 33 | 76 | F | S | 10 | N | | | | | | No information | | | | |
| 39 | 77 | F | S | 6 | N | | | | | | No information | | | | |
| 45 | 67 | M | N | ----- | N | | Dyslipidemia | | | | 0 | | | | |
| 46 | 66 | F | S | 6 | N | HBP | Dyslipidemia | Tabagism | Obesity | | 1 | ACE | | | |
| 42 | 72 | M | S | 5 | N | HBP | Dyslipidemia | | | | 3 | ACE | BB | | CCB |
| 43 | 70 | M | S | 8 | N | HBP | Dyslipidemia | | Obesity | T2DM | 2 | ACE | BB | | |
| 41 | 78 | M | N | ----- | N | HBP | Dyslipidemia | | | T2DM | 2 | | BB | ARBs | |
| 44 | 75 | F | N | ----- | N | | | | | | No information | | | | |
| 49 | 57 | M | S | 6 | N | HBP | Dyslipidemia | Tabagism | | | 2 | ACE | BB | | |
| 50 | 66 | M | N | ----- | N | HBP | Dyslipidemia | Tabagism | | T2DM | 3 | ACE | BB | | CCB |
| 51 | 61 | F | S | 5 | N | | Dyslipidemia | Tabagism | | | 0 | | | | |
| 52 | 84 | M | N | ----- | N | | | | | | 0 | | | | |
| 53 | 66 | M | N | ----- | N | HBP | | | | | 2 | ACE | BB | | |
| 54 | 74 | M | S | 7 | N | HBP | Dyslipidemia | | | T2DM | 1 | ACE | | | |
| 48 | 70 | M | N | ----- | N | | | | | | No information | | | | |
| 55 | 76 | M | S | 6 | N | HBP | Dyslipidemia | | | | 1 | | | | CCB |
| 56 | 56 | M | N | ----- | N | | | | | | No information | | | | |
| 57 | 78 | M | N | ----- | N | | | | | | No information | | | | |
| 58 | 78 | M | N | ----- | N | | | | | | No information | | | | |
| 59 | 78 | F | N | ----- | N | | | | | | No information | | | | |
| 64 | 89 | M | S | 7 | N | HBP | Dyslipidemia | | | | 1 | | | ARBs | |
| 60 | 73 | F | S | 6 | N | HBP | | Tabagism | | | 2 | | BB | | CCB |
| 61 | 81 | M | S | 10 | N | | | | | | No information | | | | |
| 62 | 41 | M | S | 6 | N | HBP | | | | | 0 | | | | |
| 65 | 68 | F | S | 9 | N | HBP | Dyslipidemia | Tabagism | | | 2 | ACE | BB | | |

APPENDIX 6: STAI-S values for Cross-sectional study.

| ID | T1 | | | | | | | | | | | | | | | | | | | | T5 | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 02 | 3 | 3 | 1 | 3 | 1 | 0 | 3 | 3 | 3 | 3 | 1 | 1 | 0 | 0 | 3 | 1 | 3 | 0 | 3 | 3 | 3 | 3 | 2 | 0 | 3 | 0 | 2 | 3 | 0 | 2 | 3 | 0 | 2 | 0 | 3 | 3 | 0 | 0 | 3 | 3 |
| 03 | 2 | 3 | 0 | 0 | 2 | 0 | 1 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 3 | 3 | 2 | 3 | 2 | 0 | 3 | 0 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 3 | 1 | 1 | 0 | 2 | 3 |
| 04 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 3 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 3 | 1 | 1 | 2 | 3 | 2 | 3 | 1 | 1 | 2 | 1 | 3 | 2 | 1 | 2 | 3 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 3 |
| 05 | 2 | 3 | 1 | 1 | 3 | 2 | 0 | 1 | 0 | 0 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 3 | 3 | 1 | 2 | 1 | 1 | 1 | 2 | 2 |
| 06 | 1 | 3 | 0 | 3 | 3 | 0 | 3 | 3 | 0 | 0 | 3 | 0 | 1 | 0 | 3 | 3 | 3 | 2 | 0 | 1 | 2 | 2 | 1 | 0 | 2 | 0 | 2 | 2 | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 1 | 2 | 3 | 3 | 2 |
| 07 | 3 | 2 | 1 | 0 | 2 | 0 | 0 | 3 | 1 | 2 | 2 | 2 | 0 | 0 | 1 | 3 | 1 | 0 | 1 | 1 | 3 | 3 | 0 | 0 | 3 | 0 | 2 | 2 | 0 | 3 | 3 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 |
| 08 | 2 | 2 | 0 | 0 | 3 | 0 | 2 | 3 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 2 | 2 | 2 | 2 | 0 | 3 | 0 | 1 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 2 | 2 |
| 09 | 1 | 2 | 2 | 2 | 3 | 1 | 2 | 3 | 2 | 3 | 3 | 2 | 1 | 2 | 0 | 3 | 2 | 2 | 3 | 3 | 2 | 3 | 2 | 0 | 3 | 0 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 3 | 1 | 1 | 0 | 2 | 3 |
| 10 | 2 | 3 | 2 | 3 | 3 | 1 | 0 | 2 | 0 | 1 | 2 | 2 | 2 | 1 | 1 | 2 | 1 | 0 | 2 | 2 | 1 | 3 | 2 | 1 | 3 | 0 | 0 | 1 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 3 | 3 |
| 11 | 2 | 2 | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 0 | 3 | 1 | 2 | 3 | 1 | 0 | 0 | 2 | 3 | 1 | 1 | 2 | 3 |
| 01 | 1 | 3 | 3 | 3 | 3 | 0 | 1 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 2 | 0 | 1 | 2 | 3 | 3 | 0 | 0 | 3 | 0 | 2 | 2 | 0 | 3 | 3 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 |
| 12 | 2 | 3 | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 1 | 3 | 2 | 3 | 1 | 0 | 3 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 2 | 2 | 0 | 0 | 3 | 2 |
| 13 | 1 | 3 | 3 | 3 | 3 | 0 | 1 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 2 | 0 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 0 | 1 | 3 | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 0 | 1 | 2 | 2 |
| 14 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 3 | 0 | 0 | 3 | 1 | 1 | 3 | 0 | 3 | 3 | 1 | 1 | 1 | 3 | 3 | 1 | 1 | 3 | 3 |
| 15 | 2 | 2 | 1 | 0 | 2 | 1 | 2 | 1 | 0 | 2 | 2 | 1 | 0 | 2 | 2 | 2 | 1 | 1 | 3 | 3 | 2 | 2 | 2 | 0 | 3 | 0 | 1 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 2 | 2 |
| 17 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 2 | 2 |
| 18 | 2 | 1 | 2 | 2 | 1 | 2 | 3 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 3 | 3 | 2 | 2 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 3 | 3 | 1 | 2 | 1 | 1 | 1 | 2 | 2 |
| 19 | 1 | 3 | 1 | 1 | 3 | 1 | 0 | 3 | 0 | 2 | 3 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 1 | 3 | 2 | 3 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 3 | 3 | 0 | 1 | 0 | 2 | 2 | 0 | 0 | 3 | 3 |
| 20 | 1 | 3 | 0 | 3 | 3 | 0 | 3 | 3 | 0 | 0 | 3 | 0 | 1 | 0 | 3 | 3 | 3 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 2 | 0 |
| 21 | 2 | 3 | 1 | 0 | 3 | 0 | 2 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 0 | 1 | 0 | 1 | 3 | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 0 | 1 | 2 | 2 |
| 22 | 2 | 3 | 1 | 3 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 1 | 1 | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 3 | 2 | 0 | 3 | 1 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 3 |
| 23 | 2 | 2 | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 1 | 0 | 3 | 0 | 1 | 2 | 0 | 2 | 3 | 0 | 1 | 0 | 2 | 2 | 0 | 0 | 2 | 1 |
| 26 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 2 | 1 | 0 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 0 | 3 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 2 | 2 | 3 | 2 |
| 27 | 2 | 3 | 1 | 0 | 3 | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 3 | 2 | 2 | 3 | 1 | 0 | 3 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 2 | 2 | 0 | 0 | 3 | 2 |
| 28 | 3 | 1 | 2 | 3 | 0 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 2 | 3 | 0 | 1 | 2 | 1 | 1 | 3 | 3 | 2 | 1 | 3 | 3 | 3 | 2 | 1 | 2 | 3 | 2 | 2 | 1 | 3 | 3 | 3 | 2 | 3 | 3 |
| 29 | 2 | 2 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 1 | 0 | 1 | 2 | 1 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 1 |
| 30 | 3 | 2 | 1 | 0 | 2 | 0 | 0 | 3 | 1 | 2 | 2 | 2 | 0 | 0 | 1 | 3 | 1 | 0 | 1 | 1 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 3 | 2 | 2 | 2 | 2 | 3 | 0 | 1 | 3 | 2 | 2 | 3 | 3 |
| 31 | 3 | 3 | 1 | 3 | 1 | 0 | 3 | 3 | 3 | 3 | 1 | 1 | 0 | 0 | 3 | 1 | 3 | 0 | 3 | 3 | 1 | 3 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |
| 24 | 3 | 3 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 3 | 2 | 3 | 2 | 1 | 0 | 3 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 2 | 1 | 0 | 3 | 2 |
| 25 | 1 | 3 | 1 | 1 | 3 | 1 | 0 | 3 | 0 | 2 | 3 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 1 | 3 | 2 | 2 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 2 | 2 |
| 34 | 3 | 3 | 1 | 1 | 1 | 0 | 3 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 3 | 2 | 3 | 0 | 0 | 3 | 1 | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 0 | 2 | 1 | 2 | 1 | 1 | 2 |
| 35 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 3 | 2 | 0 | 3 | 1 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 3 |
| 36 | 2 | 2 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 3 | 2 | 1 | 3 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 1 | 1 | 0 | 2 | 3 | 2 | 1 | 3 | 3 |
| 37 | 3 | 3 | 0 | 0 | 3 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | 2 | 2 | 2 | 3 | 2 | 0 | 3 | 0 | 0 | 2 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 2 | 2 |
| 38 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 3 | 2 | 2 | 3 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 3 | 3 | 0 | 1 | 0 | 2 | 2 | 0 | 0 | 3 | 3 |
| 40 | 2 | 2 | 1 | 1 | 3 | 2 | 3 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 0 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 |
| 32 | 1 | 3 | 3 | 3 | 3 | 0 | 1 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 2 | 0 | 1 | 2 | 2 | 3 | 1 | 0 | 2 | 0 | 1 | 2 | 0 | 2 | 3 | 1 | 1 | 0 | 2 | 2 | 1 | 0 | 3 | 2 |
| 33 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 2 | 0 | 2 | 2 | 1 | 1 | 2 | 2 | 3 | 3 | 0 | 1 | 3 | 0 | 0 | 2 | 1 | 3 | 2 | 1 | 1 | 0 | 2 | 2 | 1 | 0 | 2 | 2 |
| 39 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 2 | 0 | 0 | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 2 | 0 | 1 | 2 |
| 45 | 2 | 2 | 1 | 2 | 2 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 0 | 1 | 2 | 2 | 2 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 2 |
| 46 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 3 | 2 | 1 | 2 | 2 | 2 | 2 | 1 | 3 | 3 | 1 | 1 | 0 | 2 | 3 | 2 | 1 | 3 | 3 |
| 42 | 2 | 3 | 1 | 3 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 1 | 1 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 0 | 2 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 1 | 0 | 1 | 3 | 2 | 0 | 0 | 3 | 2 |
| 43 | 3 | 3 | 0 | 0 | 3 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | 2 | 2 | 3 | 2 | 1 | 0 | 3 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 2 | 1 | 0 | 3 | 2 |
| 41 | 1 | 3 | 2 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 2 | 1 | 1 | 2 | 2 | | |
| 44 | 1 | 1 | 2 | 2 | 3 | 1 | 3 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 1 | 0 | 3 | 0 | 3 | 2 | 1 | 1 | 1 | 2 | 2 | 0 | 2 | 2 | 2 | 2 | 1 | 1 |
| 49 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 3 | 2 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 3 | 3 |
| 50 | 2 | 3 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 3 | 3 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 2 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 3 | 3 |
| 51 | 2 | 2 | 2 | 2 | 1 | 2 | 0 | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 1 | 0 | 2 | 1 | 0 | 2 | 3 | 3 | 2 | 1 | 1 | 2 | 1 | 0 | 2 | 2 | |
| 52 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 0 | 2 | 3 | 1 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 2 | 1 | 1 | 2 | 2 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 2 |
| 53 | 1 | 3 | 3 | 3 | 3 | 0 | 1 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 2 | 0 | 1 | 2 | 2 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 |
| 54 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 3 | 3 | 2 | 2 | 3 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 |
| 48 | 0 | 3 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 0 | 1 | 3 | 0 | 3 | 1 | 1 | 3 | 2 | 2 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 3 | 2 | 1 | 1 | 3 |
| 55 | 3 | 2 | 1 | 0 | 3 | 0 | 0 | 2 | 1 | 1 | 3 | 1 | 1 | 3 | 2 | 1 | 1 | 0 | 1 | 2 | 1 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 1 | 2 | 1 | 1 | 3 | 1 | 0 | 2 | 1 | 0 | 3 | 2 |
| 56 | 1 | 3 | 3 | 3 | 3 | 0 | 1 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 2 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 3 | 3 |
| 57 | 3 | 3 | 1 | 0 | 3 | 1 | 1 | 2 | 1 | 2 | 2 | 0 | 0 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 2 |
| 58 | 3 | 3 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 1 | 2 | 2 | 0 | 1 | 3 | 0 | 0 | 2 | 3 | 3 | 3 | 2 | 0 | 3 | 0 | 0 | 2 | 0 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 0 | 0 | 2 | 2 |
| 59 | 0 | 2 | 2 | 2 | 3 | 0 | 2 | 3 | 0 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 3 | 0 | 1 | 0 | 0 | 3 | 3 | 0 | 3 | 3 | 1 | 0 | 2 | 1 | 0 | 3 | 0 | 1 | 2 |
| 64 | 2 | 3 | 1 | 2 | 2 | 1 | 2 | 3 | 0 | 2 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 1 | 0 | 3 | 3 | 3 | 3 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 |
| 60 | 2 | 1 | 1 | 0 | 3 | 1 | 2 | 2 | 0 | 1 | 2 | 1 | 1 | 0 | 2 | 3 | 1 | 0 | 3 | 3 | 2 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 2 |
| 61 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 2 | 2 | 3 | 3 | 0 | 0 | 3 | 0 | 3 | 2 | 0 | 2 | 3 | 1 | 1 | 3 | 3 | 3 | 2 | 1 | 2 | 3 |
| 62 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 2 | 2 | 1 | 0 | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 |
| 65 | 2 | 3 | 1 | 3 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 1 | 1 | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 2 | 2 |

APPENDIX 7: STAI-S statistic results to Cross-sectional study.

**Paired Samples Statistics**

| | | Std. Deviation | Std. Error Mean | t | df | p value |
|---|---|---|---|---|---|---|
| Pair 1 | STAI_S_T1 | 8,802 | 1,109 | 3,435 | 62 | 0,001 |
| | STAI_S_T5 | 9,643 | 1,215 | | | |

**Paired Samples**

| | | Sig. |
|---|---|---|
| Pair 1 | STAI_S_T1 & STAI_S_T5 | 0,000 |

**Paired Samples Test**

| | | Std. Error Mean | Interval of the | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| | | | Lower | Upper | | | |
| Pair 1 | STAI_S_T1 - STAI_S_T5 | 1,044 | 1,499 | 5,675 | 3,435 | 62 | 0,001 |

**Paired Samples Effect Sizes**

| | | Point Estimate | Interval | |
|---|---|---|---|---|
| | | | Lower | Upper |
| Pair 1 | STAI_S_T1 - STAI_S_T5 | 0,433 | 0,173 | 0,689 |
| | | 0,430 | 0,172 | 0,685 |

| | | | Mean | Std. Error | 95% Confidence Interval Min | 95% Confidence Interval Max |
|---|---|---|---|---|---|---|
| Sample Cross Selectional study | STAI_S_T1 | | 40,444 | 1,140 | 38,181 | 42,708 |
| | STAI_S_T5 | | 36,857 | 1,231 | 34,413 | 39,302 |

| | | VAS_T1 |
|---|---|---|
| **STAI_S_T1** | Pearson Correlation | **,341**** |
| | Sig. (2-tailed) | 0,006 |
| | N | 63 |

| | | VAS_T5 |
|---|---|---|
| **STAI_S_T5** | Pearson Correlation | **,430**** |
| | Sig. (2-tailed) | 0,000 |
| | N | 63 |

APPENDIX 8: VAS scores for Cross-sectional study.

| ID | VAS scores | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 |
| 02 | 8 | 5 | 2 | 2 | 2 |
| 03 | 4 | 3 | 3 | 3 | 3 |
| 04 | 5 | 4 | 4 | 5 | 3 |
| 05 | 8 | 5 | 6 | 8 | 3 |
| 06 | 5 | 5 | 5 | 7 | 5 |
| 07 | 10 | 8 | 4 | 4 | 0 |
| 08 | 4 | 4 | 4 | 4 | 4 |
| 09 | 10 | 10 | 9 | 8 | 5 |
| 10 | 8 | 2 | 4 | 4 | 4 |
| 11 | 7 | 4 | 4 | 4 | 3 |
| 01 | 0 | 0 | 0 | 0 | 0 |
| 12 | 7 | 7 | 7 | 7 | 1 |
| 13 | 2 | 1 | 1 | 1 | 1 |
| 14 | 5 | 0 | 0 | 1 | 0 |
| 15 | 5 | 4 | 4 | 6 | 3 |
| 17 | 6 | 5 | 8 | 8 | 5 |
| 18 | 2 | 2 | 3 | 4 | 2 |
| 19 | 8 | 2 | 3 | 4 | 0 |
| 20 | 10 | 0 | 5 | 8 | 3 |
| 21 | 8 | 6 | 6 | 7 | 1 |
| 22 | 8 | 0 | 3 | 8 | 1 |
| 23 | 2 | 0 | 0 | 0 | 0 |
| 26 | 6 | 2 | 2 | 4 | 4 |
| 27 | 5 | 6 | 5 | 5 | 5 |
| 28 | 9 | 6 | 8 | 8 | 9 |
| 29 | 9 | 5 | 4 | 8 | 2 |
| 30 | 9 | 9 | 7 | 10 | 2 |
| 31 | 6 | 6 | 0 | 6 | 6 |
| 16 | 3 | 9 | 3 | 6 | 2 |
| 24 | 8 | 5 | 5 | 6 | 2 |
| 25 | 5 | 4 | 3 | 3 | 1 |
| 34 | 5 | 5 | 7 | 8 | 5 |
| 35 | 1 | 1 | 1 | 9 | 3 |
| 36 | 8 | 7 | 5 | 8 | 2 |
| 37 | 5 | 0 | 0 | 0 | 0 |
| 38 | 7 | 4 | 4 | 6 | 2 |
| 40 | 2 | 2 | 3 | 3 | 3 |
| 32 | 3 | 3 | 2 | 4 | 2 |
| 33 | 5 | 0 | 2 | 2 | 1 |
| 39 | 5 | 5 | 0 | 7 | 0 |
| 45 | 3 | 3 | 4 | 5 | 4 |
| 46 | 8 | 8 | 6 | 8 | 3 |
| 42 | 3 | 3 | 3 | 3 | 3 |
| 43 | 1 | 1 | 1 | 1 | 0 |
| 41 | 2 | 1 | 2 | 2 | 1 |
| 44 | 9 | 7 | 8 | 9 | 5 |
| 49 | 3 | 0 | 0 | 0 | 0 |
| 50 | 4 | 4 | 7 | 6 | 7 |
| 51 | 9 | 8 | 8 | 9 | 3 |
| 52 | 4 | 3 | 4 | 4 | 4 |
| 53 | 5 | 0 | 0 | 0 | 0 |
| 54 | 4 | 5 | 4 | 8 | 2 |
| 48 | 0 | 0 | 0 | 0 | 0 |
| 55 | 7 | 2 | 3 | 5 | 4 |
| 56 | 2 | 0 | 0 | 0 | 0 |
| 57 | 2 | 2 | 2 | 3 | 1 |
| 58 | 5 | 4 | 6 | 5 | 5 |
| 59 | 9 | 0 | 0 | 8 | 4 |
| 64 | 5 | 5 | 5 | 2 | 2 |
| 60 | 5 | 4 | 2 | 4 | 3 |
| 61 | 3 | 3 | 3 | 3 | 3 |
| 62 | 6 | 2 | 2 | 4 | 2 |
| 65 | 4 | 5 | 4 | 3 | 3 |

APPENDIX 9: VAS statistic results to Cross-sectional study.

| | Minimum | Maximum | Mean | Standard Deviation | Median | Percentile 25 | Percentile 75 |
|---|---|---|---|---|---|---|---|
| VAS_T1 | 0 | 10 | 4,76 | 2,59 | 5,00 | 3,00 | 8,00 |
| VAS_T2 | 0 | 10 | 3,69 | 2,67 | 4,00 | 2,00 | 5,00 |
| VAS_T3 | 0 | 9 | 5,49 | 2,46 | 5,00 | 2,00 | 3,00 |
| VAS_T4 | 0 | 10 | 4,33 | 2,83 | 4,00 | 3,00 | 8,00 |
| VAS_T5 | 0 | 9 | 2,49 | 2,61 | 2,00 | 1,00 | 4,00 |

**Pairwise Comparisons**

| Sample 1-Sample 2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj. Sig.[a] |
|---|---|---|---|---|---|
| VAS_T5-VAS_T2 | 0,016 | 0,282 | 0,056 | 0,955 | 1,000 |
| VAS_T5-VAS_T3 | 0,151 | 0,282 | 0,535 | 0,592 | 1,000 |
| VAS_T5-VAS_T1 | 0,579 | 0,282 | 2,057 | 0,040 | 0,397 |
| VAS_T5-VAS_T4 | 1,119 | 0,282 | 3,972 | 0,000 | 0,001 |
| VAS_T2-VAS_T3 | -0,135 | 0,282 | -0,479 | 0,632 | 1,000 |
| VAS_T2-VAS_T1 | 0,563 | 0,282 | 2,000 | 0,045 | 0,455 |
| VAS_T2-VAS_T4 | -1,103 | 0,282 | -3,916 | 0,000 | 0,001 |
| VAS_T3-VAS_T1 | 0,429 | 0,282 | 1,521 | 0,128 | 1,000 |
| VAS_T3-VAS_T4 | -0,968 | 0,282 | -3,437 | 0,001 | 0,006 |
| VAS_T1-VAS_T4 | -0,540 | 0,282 | -1,916 | 0,055 | 0,554 |

Each row tests the null hypothesis that the Sample 1 and Sample 2

a. Significance values have been adjusted by the Bonferroni correction for

APPENDIX 10: Cortisol data for Cross-sectional study.

| ID | Cortisol Measurments | | | | Cortisol Collection time | | | |
|---|---|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | | | | |
| 02 | 5,07396351 | 2,01126398 | | 3,98731257 | 11:30 | 8:26 | 8:50 | 9:46 |
| 03 | 8,77747955 | 4,26541938 | 3,03797239 | 3,03797239 | 8:30 | 8:38 | 8:54 | 9:56 |
| 04 | | | | | 8:00 | 8:28 | 8:45 | 10:04 |
| 05 | | | | | 9:10 | 8:40 | 8:54 | 10:09 |
| 06 | | | | | 9:00 | 9:03 | 9:10 | 10:15 |
| 07 | 5,18341205 | 4,09577179 | 4,69655543 | | 8:00 | 8:48 | 9:05 | 10:16 |
| 08 | | | | | 8:20 | 8:55 | 9:10 | 10:16 |
| 09 | 1,84803529 | 5,11012229 | 2,94451208 | 2,46708998 | 9:30 | 9:06 | 9:32 | 10:18 |
| 10 | 4,15137415 | 7,96507056 | 6,60763658 | 4,17952675 | 11:00 | 9:10 | 9:16 | 10:24 |
| 11 | 1,45559371 | 5,33398177 | 10,6562337 | 4,01408997 | 7:00 | 9:16 | 9:28 | 10:30 |
| 01 | 5,99405766 | 5,37247717 | 4,96738963 | 4,12345663 | 9:20 | 8:50 | 9:03 | 10:33 |
| 12 | | 6,86465306 | 3,61099653 | 2,68461596 | 9:15 | 9:10 | 9:25 | 10:33 |
| 13 | 1,96604978 | 2,71758817 | 1,06975868 | 4,01408997 | 9:00 | 9:05 | 9:22 | 10:36 |
| 14 | 1,41789235 | | 2,21836686 | 3,11538466 | 9:00 | 8:55 | 9:10 | 10:37 |
| 15 | 2,46708998 | 4,68433241 | 4,93944862 | 6,37107933 | 9:30 | 9:22 | 9:29 | 10:42 |
| 17 | | | | | 9:00 | 9:20 | 9:35 | 10:50 |
| 18 | | | | | 8:40 | 9:00 | 9:28 | 10:57 |
| 19 | | 1,28365961 | 1,74279376 | 0,93559966 | 9:20 | 9:22 | 9:45 | 11:00 |
| 20 | | 3,24101173 | 4,68433241 | 0,62506714 | 10:30 | 9:51 | 10:03 | 11:11 |
| 21 | | 6,85674182 | 7,37132418 | 1,88544943 | 11:00 | 9:50 | 10:08 | 11:12 |
| 22 | | 6,69173031 | 7,02496554 | 4,93944862 | 8:30 | 9:50 | 10:00 | 11:14 |
| 23 | | | | | 10:00 | 9:40 | 10:00 | 11:23 |
| 26 | | 4,31927883 | 2,8632 | 4,4386756 | 10:00 | 9:40 | 9:58 | 11:25 |
| 27 | | 4,20211025 | 9,1082321 | 12,7904309 | 9:40 | 9:55 | 10:10 | 11:27 |
| 28 | | 6,06249927 | 2,8632 | 0,30526231 | 10:30 | 10:20 | 10:43 | 11:30 |
| 29 | | | | | 10:00 | 10:21 | 10:34 | 11:34 |
| 30 | | 2,03355266 | 4,4386756 | 1,53860094 | 12:10 | 9:45 | 10:10 | 11:39 |
| 31 | | 7,73137275 | 0,39225586 | 1,22287163 | 9:30 | 10:15 | 10:28 | 11:39 |
| 16 | 4,72938437 | 5,91258214 | 5,62119903 | 3,64820474 | 10:00 | 10:22 | 10:42 | 11:41 |
| 24 | | 11,4375184 | 5,07067372 | 4,4386756 | 9:30 | 9:50 | 10:10 | 11:43 |
| 25 | 4,50543483 | 1,94391388 | 8,15884355 | 4,44388816 | 10:00 | 10:15 | 10:37 | 11:47 |
| 34 | | 2,77308204 | 6,06249927 | 0,82811225 | 10:00 | 10:12 | 10:26 | 11:49 |
| 35 | | 8,69537219 | 1,10468807 | 1,47305467 | 11:00 | 10:20 | 10:47 | 11:50 |
| 36 | | 9,98457618 | 4,81068442 | 6,06249927 | 10:00 | 10:37 | 10:55 | 11:50 |
| 37 | | 1,67344763 | 6,21531155 | 0,14114649 | 10:20 | 10:29 | 11:10 | 11:52 |
| 38 | | 2,18730639 | 3,14389147 | 4,68433241 | 10:30 | 10:42 | 10:55 | 11:53 |
| 40 | | | | | 10:20 | 10:20 | 10:30 | 12:02 |
| 32 | | 1,88544943 | 0,57669224 | 0,14114649 | 11:00 | 10:34 | 10:53 | 12:02 |
| 33 | | 1,43 | 4,20211025 | 3,54352721 | 10:20 | 10:22 | 10:32 | 12:07 |
| 39 | | 4,56034498 | 4,68433241 | 2,77308204 | 10:40 | 10:36 | 11:10 | 12:07 |
| 45 | | 11,4375184 | 4,31927883 | 4,31927883 | 10:30 | 10:50 | 11:07 | 12:10 |
| 46 | | 6,52986446 | 1,88544943 | 3,24101173 | 20:00 | 10:20 | 10:35 | 12:12 |
| 42 | | | | | 10:10 | 10:40 | 10:58 | 12:15 |
| 43 | | 1,88544943 | 3,33996735 | 0,72464733 | 11:00 | 10:47 | 11:05 | 12:17 |
| 41 | | 3,86354444 | | 6,21531155 | 11:00 | 11:05 | 11:15 | 12:20 |
| 44 | | 2,34692227 | 1,40873008 | 0,14114649 | 11:00 | 10:52 | 11:25 | 12:23 |
| 49 | | 4,20211025 | 7,37132418 | 0,06380599 | 10:30 | 10:44 | 10:52 | 12:25 |
| 50 | | 1,22287163 | 2,26636798 | 1,67344763 | 12:00 | 11:02 | 11:18 | 12:26 |
| 51 | | | | | 10:40 | 10:55 | 11:15 | 12:30 |
| 52 | | 3,14389147 | 1,47305467 | 2,34692227 | 10:40 | 10:55 | 11:06 | 12:32 |
| 53 | | | | | 11:30 | 11:19 | 11:29 | 12:35 |
| 54 | 2,0696028 | 5,99405766 | 3,34116936 | 2,70104012 | 11:30 | 11:05 | 11:30 | 12:43 |
| 48 | | 2,8632 | 1,67344763 | 1,53860094 | 11:20 | 11:10 | 11:33 | 12:46 |
| 55 | 4,86359804 | 4,35351666 | 3,78087248 | 7,59479597 | 8:30 | 11:00 | 11:13 | 12:47 |
| 56 | 1,4479534 | 2,39469776 | 3,63467769 | 3,17504461 | 10:30 | 11:26 | 11:46 | 12:48 |
| 57 | 1,75794706 | 10,1198165 | 11,2299834 | 7,71573714 | 11:00 | 11:09 | 11:22 | 12:49 |
| 58 | | 6,65805305 | 5,65193481 | 3,05710102 | 11:20 | 11:20 | 11:37 | 13:00 |
| 59 | 4,8296037 | 4,50543483 | 3,09580576 | 3,40622222 | 11:00 | 11:20 | 11:42 | 13:04 |
| 64 | 1,40315675 | 2,51182018 | 2,63607591 | 3,23612014 | 11:00 | 11:04 | 11:18 | 13:13 |
| 60 | | 3,07637835 | 3,29865185 | 1,89 | 10:30 | 11:53 | 12:04 | 13:15 |
| 61 | 7,71573714 | 3,4504611 | 5,07396351 | 1,97723457 | 11:20 | 11:11 | 11:29 | 13:32 |
| 62 | | 6,97099802 | 7,53522972 | 1,57651238 | 15:00 | 12:00 | 12:22 | 13:45 |
| 65 | 4,35351666 | 1,2723923 | 4,69655543 | 1,55134997 | 7:00 | 12:15 | 12:35 | 14:05 |

APPENDIX 11: Cortisol concentrations and standards.

| Reading Nº | Sample | Absorvance(nm) | 1/Abs(nm) | Concentration (pg/mL) | Concentration (pg/mL) | Concentration (ng/mL) | Concentration (g/L) | Concentration (mol/L) | Concentration (nmol/L) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 15.1 | 0,233 | 4,292 | 424,63 | 1698,51 | 1,699 | 0,0000016985119328 | 0,00000000468606724 | 4,686 |
| 11 | 15.2 | 0,231 | 4,329 | 447,75 | 1791,00 | 1,791 | 0,0000017909952527 | 0,00000000494122180 | 4,941 |
| 12 | 15.3 | 0,221 | 4,525 | 577,49 | 2309,98 | 2,310 | 0,0000023099797318 | 0,00000000637306112 | 6,373 |
| 13 | 16.1 | 0,224 | 4,464 | 535,94 | 2143,77 | 2,144 | 0,0000021437690306 | 0,00000000591449824 | 5,914 |
| 14 | 16.2 | 0,226 | 4,425 | 509,53 | 2038,14 | 2,038 | 0,0000020381389866 | 0,00000000562307285 | 5,623 |
| 15 | 16.3 | 0,242 | 4,132 | 330,72 | 1322,90 | 1,323 | 0,0000013228989878 | 0,00000000364977925 | 3,650 |
| 16 | 19.1 | 0,271 | 3,690 | 116,42 | 465,70 | 0,466 | 0,0000004656985618 | 0,00000000128482746 | 1,285 |
| 17 | 19.2 | 0,264 | 3,788 | 158,04 | 632,15 | 0,632 | 0,0000006321472176 | 0,00000000174404684 | 1,744 |
| 18 | 19.3 | 0,277 | 3,610 | 84,88 | 339,52 | 0,340 | 0,0000003395162392 | 0,00000000093669988 | 0,937 |
| 19 | 20.1 | 0,246 | 4,065 | 293,82 | 1175,28 | 1,175 | 0,0000011752841245 | 0,00000000324252090 | 3,243 |
| 20 | 20.2 | 0,233 | 4,292 | 424,63 | 1698,51 | 1,699 | 0,0000016985119328 | 0,00000000468606724 | 4,686 |
| 21 | 20.3 | 0,283 | 3,534 | 56,73 | 226,94 | 0,227 | 0,0000002269377607 | 0,00000000062610429 | 0,626 |
| 22 | 21.1 | 0,218 | 4,587 | 621,51 | 2486,04 | 2,486 | 0,0000024860378150 | 0,00000000685879218 | 6,859 |
| 23 | 21.2 | 0,215 | 4,651 | 668,14 | 2672,58 | 2,673 | 0,0000026725792969 | 0,00000000737344616 | 7,373 |
| 24 | 21.3 | 0,262 | 3,817 | 170,97 | 683,86 | 0,684 | 0,0000006838635091 | 0,00000000188672822 | 1,887 |
| 25 | 22.1 | 0,219 | 4,566 | 606,55 | 2426,22 | 2,426 | 0,0000024262193382 | 0,00000000669375749 | 6,694 |
| 26 | 22.2 | 0,217 | 4,608 | 636,76 | 2547,02 | 2,547 | 0,0000025470207123 | 0,00000000702703943 | 7,027 |
| 27 | 22.3 | 0,231 | 4,329 | 447,75 | 1791,00 | 1,791 | 0,0000017909952527 | 0,00000000494122180 | 4,941 |
| 28 | 24.1 | 0,196 | 5,102 | 1036,65 | 4146,61 | 4,147 | 0,0000041466067805 | 0,00000001144017762 | 11,440 |
| 29 | 24.2 | 0,230 | 4,348 | 459,64 | 1838,57 | 1,839 | 0,0000018385662004 | 0,00000000507246648 | 5,072 |
| 30 | 24.3 | 0,235 | 4,255 | 402,36 | 1609,46 | 1,609 | 0,0000016094576369 | 0,00000000444037311 | 4,440 |
| 31 | 26.1 | 0,236 | 4,237 | 391,54 | 1566,17 | 1,566 | 0,0000015661744556 | 0,00000000432095805 | 4,321 |
| 32 | 26.2 | 0,250 | 4,000 | 259,58 | 1038,32 | 1,038 | 0,0000010383200000 | 0,00000000286464713 | 2,865 |
| 33 | 26.3 | 0,235 | 4,255 | 402,36 | 1609,46 | 1,609 | 0,0000016094576369 | 0,00000000444037311 | 4,440 |
| 34 | 27.1 | 0,237 | 4,219 | 380,92 | 1523,70 | 1,524 | 0,0000015236989927 | 0,00000000420377143 | 4,204 |
| 35 | 27.2 | 0,206 | 4,854 | 825,56 | 3302,22 | 3,302 | 0,0000033022241418 | 0,00000000911058915 | 9,111 |
| 36 | 27.3 | 0,191 | 5,236 | 1159,26 | 4637,05 | 4,637 | 0,0000046370451271 | 0,00000001279326030 | 12,793 |
| 37 | 28.1 | 0,223 | 4,484 | 549,53 | 2198,12 | 2,198 | 0,0000021981158181 | 0,00000000606443695 | 6,064 |
| 38 | 28.2 | 0,250 | 4,000 | 259,58 | 1038,32 | 1,038 | 0,0000010383200000 | 0,00000000286464713 | 2,865 |
| 39 | 28.3 | 0,290 | 3,448 | 27,75 | 111,00 | 0,111 | 0,0000001109965279 | 0,00000000030623111 | 0,306 |
| 40 | 30.1 | 0,260 | 3,846 | 184,39 | 737,55 | 0,738 | 0,0000007375545562 | 0,00000000203485779 | 2,035 |
| 41 | 30.2 | 0,235 | 4,255 | 402,36 | 1609,46 | 1,609 | 0,0000016094576369 | 0,00000000444037311 | 4,440 |
| 42 | 30.3 | 0,267 | 3,745 | 139,53 | 558,12 | 0,558 | 0,0000005581219307 | 0,00000000153981662 | 1,540 |
| 43 | 31.1 | 0,213 | 4,695 | 700,78 | 2803,10 | 2,803 | 0,0000028031004589 | 0,00000000773354428 | 7,734 |
| 44 | 31.2 | 0,288 | 3,472 | 35,63 | 142,54 | 0,143 | 0,0000001425350926 | 0,00000000039324365 | 0,393 |
| 45 | 31.3 | 0,272 | 3,676 | 110,92 | 443,66 | 0,444 | 0,0000004436611419 | 0,00000000122402787 | 1,224 |
| 46 | 32.1 | 0,262 | 3,817 | 170,97 | 683,86 | 0,684 | 0,0000006838635091 | 0,00000000188672822 | 1,887 |
| 47 | 32.2 | 0,284 | 3,521 | 52,35 | 209,40 | 0,209 | 0,0000002094001349 | 0,00000000057771929 | 0,578 |
| 48 | 32.3 | 0,294 | 3,401 | 12,87 | 51,50 | 0,051 | 0,0000000514977981 | 0,00000000014207857 | 0,142 |
| 49 | 33.1 | 0,157 | 6,369 | 2447,69 | 9790,78 | 9,791 | 0,0000097907789817 | 0,00000002701202610 | 27,012 |
| 50 | 33.2 | 0,237 | 4,219 | 380,92 | 1523,70 | 1,524 | 0,0000015236989927 | 0,00000000420377143 | 4,204 |
| 51 | 33.3 | 0,243 | 4,115 | 321,24 | 1284,95 | 1,285 | 0,0000012849515338 | 0,00000000354508507 | 3,545 |
| 52 | 34.1 | 0,251 | 3,984 | 251,41 | 1005,65 | 1,006 | 0,0000010056504005 | 0,00000000277451415 | 2,775 |
| 53 | 34.2 | 0,223 | 4,484 | 549,53 | 2198,12 | 2,198 | 0,0000021981158181 | 0,00000000606443695 | 6,064 |
| 54 | 34.3 | 0,279 | 3,584 | 75,14 | 300,55 | 0,301 | 0,0000003005485551 | 0,00000000082919096 | 0,829 |
| 55 | 35.1 | 0,208 | 4,808 | 788,14 | 3152,56 | 3,153 | 0,0000031525589941 | 0,00000000869767421 | 8,698 |
| 56 | 35.2 | 0,274 | 3,650 | 100,20 | 400,82 | 0,401 | 0,0000004008160648 | 0,00000000110582151 | 1,106 |
| 57 | 35.3 | 0,268 | 3,731 | 133,59 | 534,36 | 0,534 | 0,0000005343596168 | 0,00000000147425817 | 1,474 |
| 58 | 36.1 | 0,202 | 4,950 | 904,98 | 3619,91 | 3,620 | 0,0000036199055857 | 0,00000000998704846 | 9,987 |
| 59 | 36.2 | 0,232 | 4,310 | 436,08 | 1744,32 | 1,744 | 0,0000017443163853 | 0,00000000481243830 | 4,812 |
| 60 | 36.3 | 0,223 | 4,484 | 549,53 | 2198,12 | 2,198 | 0,0000021981158181 | 0,00000000606443695 | 6,064 |
| 61 | 37.1 | 0,265 | 3,774 | 151,75 | 607,01 | 0,607 | 0,0000006070074475 | 0,00000000167468810 | 1,675 |
| 62 | 37.2 | 0,222 | 4,505 | 563,38 | 2253,51 | 2,254 | 0,0000022535120916 | 0,00000000621727112 | 6,217 |
| 63 | 37.3 | 0,294 | 3,401 | 12,87 | 51,50 | 0,051 | 0,0000000514977981 | 0,00000000014207857 | 0,142 |
| 64 | 38.1 | 0,258 | 3,876 | 198,32 | 793,29 | 0,793 | 0,0000007932939174 | 0,00000000218863852 | 2,189 |
| 65 | 38.2 | 0,247 | 4,049 | 285,02 | 1140,08 | 1,140 | 0,0000011400761842 | 0,00000000314538483 | 3,145 |
| 66 | 38.3 | 0,233 | 4,292 | 424,63 | 1698,51 | 1,699 | 0,0000016985119328 | 0,00000000468606724 | 4,686 |
| 67 | 39.1 | 0,234 | 4,274 | 413,39 | 1653,56 | 1,654 | 0,0000016535646373 | 0,00000000456206102 | 4,562 |
| 68 | 39.2 | 0,233 | 4,292 | 424,63 | 1698,51 | 1,699 | 0,0000016985119328 | 0,00000000468606724 | 4,686 |
| 69 | 39.3 | 0,251 | 3,984 | 251,41 | 1005,65 | 1,006 | 0,0000010056504005 | 0,00000000277451415 | 2,775 |
| 70 | 41.1 | 0,240 | 4,167 | 350,24 | 1400,96 | 1,401 | 0,0000014009633333 | 0,00000000386515294 | 3,865 |
| 71 | 41.2 | 0,142 | **7,042** | | | | **OUT OF STANDARDS** | | |
| 72 | 41.3 | 0,222 | 4,505 | 563,38 | 2253,51 | 2,254 | 0,0000022535120916 | 0,00000000621727112 | 6,217 |
| 73 | 43.1 | 0,262 | 3,817 | 170,97 | 683,86 | 0,684 | 0,0000006838635091 | 0,00000000188672822 | 1,887 |
| 74 | 43.2 | 0,245 | 4,082 | 302,79 | 1211,16 | 1,211 | 0,0000012111573844 | 0,00000000334149254 | 3,341 |
| 75 | 43.3 | 0,281 | 3,559 | 65,76 | 263,04 | 0,263 | 0,0000002630390405 | 0,00000000072570502 | 0,726 |
| 76 | 44.1 | 0,256 | 3,906 | 212,79 | 851,16 | 0,851 | 0,0000008511583203 | 0,00000000234828207 | 2,348 |
| 77 | 44.2 | 0,269 | 3,717 | 127,76 | 511,04 | 0,511 | 0,0000005110401692 | 0,00000000140992156 | 1,410 |
| 78 | 44.3 | 0,294 | 3,401 | 12,87 | 51,50 | 0,051 | 0,0000000514977981 | 0,00000000014207857 | 0,142 |
| 79 | 45.1 | 0,196 | 5,102 | 1036,65 | 4146,61 | 4,147 | 0,0000041466067805 | 0,00000001144017762 | 11,440 |
| 80 | 45.2 | 0,236 | 4,237 | 391,54 | 1566,17 | 1,566 | 0,0000015661744556 | 0,00000000432095805 | 4,321 |
| 81 | 45.3 | 0,236 | 4,237 | 391,54 | 1566,17 | 1,566 | 0,0000015661744556 | 0,00000000432095805 | 4,321 |
| 82 | 46.1 | 0,220 | 4,545 | 591,89 | 2367,54 | 2,368 | 0,0000023675411570 | 0,00000000653186878 | 6,532 |
| 83 | 46.2 | 0,262 | 3,817 | 170,97 | 683,86 | 0,684 | 0,0000006838635091 | 0,00000000188672822 | 1,887 |
| 84 | 46.3 | 0,246 | 4,065 | 293,82 | 1175,28 | 1,175 | 0,0000011752841245 | 0,00000000324252090 | 3,243 |
| 85 | 48.1 | 0,250 | 4,000 | 259,58 | 1038,32 | 1,038 | 0,0000010383200000 | 0,00000000286464713 | 2,865 |
| 86 | 48.2 | 0,265 | 3,774 | 151,75 | 607,01 | 0,607 | 0,0000006070074475 | 0,00000000167468810 | 1,675 |
| 87 | 48.3 | 0,267 | 3,745 | 139,53 | 558,12 | 0,558 | 0,0000005581219307 | 0,00000000153981662 | 1,540 |
| 88 | 49.1 | 0,237 | 4,219 | 380,92 | 1523,70 | 1,524 | 0,0000015236989927 | 0,00000000420377143 | 4,204 |
| 89 | 49.2 | 0,215 | 4,651 | 668,14 | 2672,58 | 2,673 | 0,0000026725792969 | 0,00000000737344616 | 7,373 |
| 90 | 49.3 | 0,296 | 3,378 | 5,86 | 23,46 | 0,023 | 0,0000000234585245 | 0,00000000006472031 | 0,065 |
| 91 | 50.1 | 0,272 | 3,676 | 110,92 | 443,66 | 0,444 | 0,0000004436611419 | 0,00000000122402787 | 1,224 |
| 92 | 50.2 | 0,257 | 3,891 | 205,49 | 821,96 | 0,822 | 0,0000008219555651 | 0,00000000226771386 | 2,268 |
| 93 | 50.3 | 0,265 | 3,774 | 151,75 | 607,01 | 0,607 | 0,0000006070074475 | 0,00000000167468810 | 1,675 |
| 94 | 52.1 | 0,247 | 4,049 | 285,02 | 1140,08 | 1,140 | 0,0000011400761842 | 0,00000000314538483 | 3,145 |
| 95 | 52.2 | 0,268 | 3,731 | 133,59 | 534,36 | 0,534 | 0,0000005343596168 | 0,00000000147425817 | 1,474 |
| 96 | 52.3 | 0,256 | 3,906 | 212,79 | 851,16 | 0,851 | 0,0000008511583203 | 0,00000000234828207 | 2,348 |

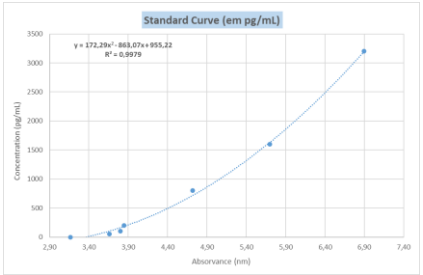
| Reading nº | Standards | 1º Absorvance (nm) | Duplicates | Absorvance (nm) | 1/Abs (nm) | Concentration (pg/mL) | Concentration (ng/mL) | Concentration (g/L) | Concentration (mol/L) | Concentration (nmol/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | S1 | 0,145 | ------ | 0,145 | 6,897 | 3200 | 3,2 | 0,0000032000000 | 8,8286E-09 | 8,829 |
| 2 e 9 | S2 | 0,166 | 0,185 | 0,176 | 5,698 | 1600 | 1,6 | 0,0000016000000 | 4,4143E-09 | 4,414 |
| 3 | S3 | 0,212 | ------ | 0,212 | 4,717 | 800 | 0,8 | 0,0000008000000 | 2,2071E-09 | 2,207 |
| 5 | S5 | 0,260 | ------ | 0,260 | 3,846 | 200 | 0,2 | 0,0000002000000 | 5,5179E-10 | 0,552 |
| 6 | S6 | 0,263 | ------ | 0,263 | 3,802 | 100 | 0,1 | 0,0000001000000 | 2,7589E-10 | 0,276 |
| 7 | S7 | 0,273 | ------ | 0,273 | 3,663 | 50 | 0,1 | 0,0000000500000 | 1,3795E-10 | 0,138 |
| 8 | S8 | 0,316 | ------ | 0,316 | 3,165 | 0 | 0,0 | 0,0000000000000 | 0,0000E+00 | 0,000 |

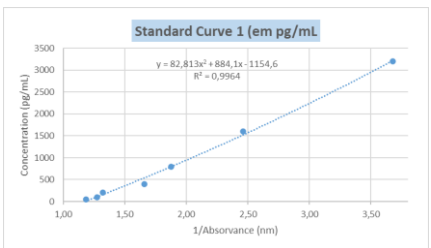
| Reading Nº | Standards | 1º Absorvance (nm) | 1/Abs (nm) | Concentration (pg/mL) | Concentration (ng/mL) | Concentration (g/L) | Concentration (mol/L) | Concentration (nmol/L) |
|---|---|---|---|---|---|---|---|---|
| 1 | S1 | 0,181 | 5,525 | 3200 | 3,2 | 0,0000032 | 0,0000000088285604 | 8,829 |
| 4 | S4 | 0,418 | 2,392 | 400 | 0,4 | 0,0000004 | 0,0000000011035700 | 1,104 |
| 5 | S5 | 0,498 | 2,008 | 200 | 0,2 | 0,0000002 | 0,0000000005517850 | 0,552 |
| 6 | S6 | 0,674 | 1,484 | 100 | 0,1 | 0,0000001 | 0,0000000002758925 | 0,276 |

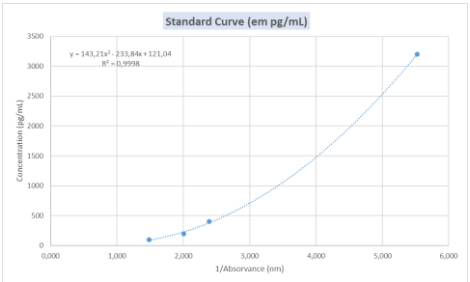
| Reading Nº | Standard | 1º Absorvance (nm) | Duplicates | Absorvance (nm) | 1/Abs (nm) | Concentration (pg/mL) | Concentration (ng/mL) | Concentration (g/L) | Concentration (mol/L) | Concentration (nmol/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | S1 | 0,272 | ------ | 0,272 | 3,676 | 3200 | 3,2 | 0,000003200 | 8,8286E-09 | 8,829 |
| 2 | S2 | 0,406 | ------ | 0,406 | 2,463 | 1600 | 1,6 | 0,000001600 | 4,4143E-09 | 4,414 |
| 3 | S3 | 0,533 | ------ | 0,533 | 1,876 | 800 | 0,8 | 0,000000800 | 2,2071E-09 | 2,207 |
| 4 | S4 | 0,603 | ------ | 0,603 | 1,658 | 400 | 0,4 | 0,000000400 | 1,1036E-09 | 1,104 |
| 5,41 | S5 | 0,844 | 0,757 | 0,757 | 1,321 | 200 | 0,2 | 0,000000200 | 5,5179E-10 | 0,552 |
| 6, 67 | S6 | 0,784 | 0,735 | 0,784 | 1,276 | 100 | 0,1 | 0,000000100 | 2,7589E-10 | 0,276 |
| 7, 68 | S7 | 0,844 | 0,637 | 0,844 | 1,185 | 50 | 0,1 | 0,000000050 | 1,3795E-10 | 0,138 |

| Reading Nº | Samples | Absorvance(nm) | 1/Abs(nm) | Concentration (pg/mL) | Concentration (pg/mL) | Concentration (ng/mL) | Concentration (g/L) | Concentration (mol/L) | Concentration (nmol/L) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 47.2 | 0,733 | 1,364 | 205,67 | 822,68 | 0,823 | 0,0000008226814292 | 0,0000000226971646 | 2,270 |
| 11 | 47.3 | 0,834 | 1,199 | 24,53 | 98,13 | 0,098 | 0,0000000981283600 | 0,0000000027072880 | 0,271 |
| 12 | 66.2 | 0,651 | 1,536 | 398,87 | 1595,48 | 1,595 | 0,0000015954796275 | 0,0000000440180883 | 4,402 |
| 13 | 66.3 | 0,777 | 1,287 | 120,41 | 481,63 | 0,482 | 0,0000004816279927 | 0,0000000132877557 | 1,329 |
| 14 | 67.2 | 0,858 | | OUT OF STANDARDS | | | | | |
| 15 | 67.3 | 0,733 | 1,364 | 205,67 | 822,68 | 0,823 | 0,0000008226814292 | 0,0000000226971646 | 2,270 |
| 16 | 68.2 | 0,818 | 1,222 | 49,97 | 199,88 | 0,200 | 0,0000001998806176 | 0,0000000055145566 | 0,551 |
| 17 | 68.3 | 0,799 | 1,252 | 81,63 | 326,51 | 0,327 | 0,0000003265101740 | 0,0000000090081712 | 0,901 |
| 18 | 69.2 | 0,509 | 1,965 | 901,98 | 3607,91 | 3,608 | 0,0000036079060587 | 0,0000000995394267 | 9,954 |
| 19 | 69.3 | 0,592 | 1,689 | 575,11 | 2300,43 | 2,300 | 0,0000023004298941 | 0,0000000634671383 | 6,347 |
| 20 | 70.2 | 0,934 | | OUT OF STANDARDS | | | | | |
| 21 | 70.3 | 0,965 | | | | | | | |
| 22 | 71.2 | 0,854 | | | | | | | |
| 23 | 71.3 | 0,699 | 1,431 | 279,70 | 1118,79 | 1,119 | 0,0000011187876030 | 0,0000000308665122 | 3,087 |
| 24 | 72.2 | 0,576 | 1,736 | 629,90 | 2519,60 | 2,520 | 0,0000025196039545 | 0,0000000695139865 | 6,951 |
| 25 | 72.3 | 0,559 | 1,789 | 691,99 | 2767,97 | 2,768 | 0,0000027679678112 | 0,0000000763661593 | 7,637 |
| 26 | 73.2 | 0,699 | 1,431 | 279,70 | 1118,79 | 1,119 | 0,0000011187876030 | 0,0000000308665122 | 3,087 |
| 27 | 73.3 | 0,659 | 1,517 | 377,67 | 1510,67 | 1,511 | 0,0000015106721906 | 0,0000000416783146 | 4,168 |
| 28 | 74.2 | 0,879 | OUT OF STANDARDS | | | | | | |
| 29 | 74.3 | 0,695 | 1,439 | 288,93 | 1155,73 | 1,156 | 0,0000011557317737 | 0,0000000318857743 | 3,189 |
| 30 | 75.2 | 0,669 | 1,495 | 351,96 | 1407,83 | 1,408 | 0,0000014078256988 | 0,0000000388408569 | 3,884 |
| 31 | 75.3 | 0,693 | 1,443 | 293,60 | 1174,38 | 1,174 | 0,0000011743808283 | 0,0000000324002877 | 3,240 |
| 32 | 76.2 | 0,946 | | | | | | | |
| 33 | 76.3 | 0,879 | | OUT OF STANDARDS | | | | | |
| 34 | 77.2 | 0,463 | 2,160 | 1141,21 | 4564,86 | 4,565 | 0,0000045648550415 | 0,0000001259409326 | 12,594 |
| 35 | 77.3 | 0,726 | 1,377 | 220,29 | 881,15 | 0,881 | 0,0000008811454166 | 0,0000000243101423 | 2,431 |
| 36 | 78.2 | 0,431 | 2,320 | 1342,48 | 5369,92 | 5,370 | 0,0000053699204763 | 0,0000001481520851 | 14,815 |
| 37 | 78.3 | 0,465 | 2,151 | 1129,68 | 4518,74 | 4,519 | 0,0000045187395537 | 0,0000001246686408 | 12,467 |
| 38 | 79.2 | 0,748 | 1,337 | 175,36 | 701,45 | 0,701 | 0,0000007014532986 | 0,0000000193525713 | 1,935 |
| 39 | 79.3 | 0,783 | 1,277 | 109,59 | 438,38 | 0,438 | 0,0000004383751175 | 0,0000000120944412 | 1,209 |
| 40 | 80.2 | 0,562 | 1,779 | 680,73 | 2722,91 | 2,723 | 0,0000027229102671 | 0,0000000751230554 | 7,512 |
| 42 | 81.2 | 0,618 | 1,618 | 492,81 | 1971,25 | 1,971 | 0,0000019712544862 | 0,0000000543854353 | 5,439 |
| 43 | 81.3 | 0,699 | 1,431 | 279,70 | 1118,79 | 1,119 | 0,0000011187876030 | 0,0000000308665122 | 3,087 |
| 44 | 82.2 | 0,512 | 1,953 | 888,06 | 3552,26 | 3,552 | 0,0000035522573486 | 0,0000000980041204 | 9,800 |
| 45 | 82.3 | 0,548 | 1,825 | 734,49 | 2937,94 | 2,938 | 0,0000029379402419 | 0,0000000810555714 | 8,106 |
| 46 | 83.2 | 0,367 | 2,725 | 1869,24 | 7476,95 | 7,477 | 0,0000074769515135 | 0,0000002062834937 | 20,628 |
| 47 | 83.3 | 0,513 | 1,949 | 883,47 | 3533,87 | 3,534 | 0,0000035338717341 | 0,0000000974968751 | 9,750 |
| 48 | 84.2 | 0,850 | | OUT OF STANDARDS | | | | | |
| 49 | 84.3 | 0,608 | 1,645 | 523,53 | 2094,14 | 2,094 | 0,0000020941367902 | 0,0000000577756660 | 5,778 |
| 50 | 85.2 | 0,563 | 1,776 | 677,00 | 2708,01 | 2,708 | 0,0000027080111001 | 0,0000000747119986 | 7,471 |
| 51 | 85.3 | 0,550 | 1,818 | 726,62 | 2906,47 | 2,906 | 0,0000029064661157 | 0,0000000801872239 | 8,019 |
| 52 | 86.2 | 0,323 | 3,096 | 2376,32 | 9505,28 | 9,505 | 0,0000095052779802 | 0,0000002622435022 | 26,224 |
| 53 | 86.3 | 0,499 | 2,004 | 949,72 | 3798,90 | 3,799 | 0,0000037988979225 | 0,0000001048087492 | 10,481 |
| 54 | 87.2 | 0,522 | 1,916 | 843,00 | 3371,99 | 3,372 | 0,0000033719876925 | 0,0000000930306156 | 9,303 |
| 55 | 87.3 | 0,473 | 2,114 | 1084,68 | 4338,73 | 4,339 | 0,0000043387275963 | 0,0000001197022457 | 11,970 |
| 56 | 88.2 | 0,541 | 1,848 | 762,54 | 3050,17 | 3,050 | 0,0000030501688514 | 0,0000000841518747 | 8,415 |
| 57 | 88.3 | 0,607 | 1,647 | 526,67 | 2106,67 | 2,107 | 0,0000021066740265 | 0,0000000581215590 | 5,812 |
| 58 | 89.2 | 0,660 | 1,515 | 375,06 | 1500,23 | 1,500 | 0,0000015002317723 | 0,0000000413902713 | 4,139 |
| 59 | 89.3 | 0,655 | 1,527 | 388,20 | 1552,79 | 1,553 | 0,0000015527881592 | 0,0000000428402626 | 4,284 |
| 60 | 90.2 | 0,743 | 1,346 | 185,32 | 741,26 | 0,741 | 0,0000007412640878 | 0,0000000204509211 | 2,045 |
| 61 | 90.3 | 0,572 | 1,748 | 644,14 | 2576,55 | 2,577 | 0,0000025765508533 | 0,0000000710851088 | 7,109 |
| 62 | 91.2 | 0,511 | 1,957 | 892,68 | 3570,72 | 3,571 | 0,0000035707245821 | 0,0000000985136176 | 9,851 |
| 63 | 91.3 | 0,730 | 1,370 | 211,90 | 847,59 | 0,848 | 0,0000008475861137 | 0,0000000233842662 | 2,338 |
| 64 | 92.2 | 0,762 | 1,312 | 148,26 | 593,04 | 0,593 | 0,0000005930355784 | 0,0000000163614076 | 1,636 |
| 65 | 92.3 | 0,618 | 1,618 | 492,81 | 1971,25 | 1,971 | 0,0000019712544862 | 0,0000000543854353 | 5,439 |
| 66 | 94.2 | 0,614 | 1,629 | 504,97 | 2019,87 | 2,020 | 0,0000020198709631 | 0,0000000557267274 | 5,573 |
| 69 | 95.3 | 0,699 | 1,431 | 279,70 | 1118,79 | 1,119 | 0,0000011187876030 | 0,0000000308665122 | 3,087 |
| 70 | 96.2 | 0,701 | 1,427 | 275,12 | 1100,49 | 1,100 | 0,0000011004902749 | 0,0000000303617027 | 3,036 |
| 71 | 96.3 | 0,619 | 1,616 | 489,80 | 1959,21 | 1,959 | 0,0000019592099342 | 0,0000000540531351 | 5,405 |
| 72 | 19.0 | 0,777 | 1,287 | 120,41 | 481,63 | 0,482 | 0,0000004816279927 | 0,0000000132877557 | 1,329 |
| 73 | 20.0 | 0,740 | 1,351 | 191,36 | 765,43 | 0,765 | 0,0000007654349160 | 0,0000000211177762 | 2,112 |
| 74 | 21.0 | 0,608 | 1,645 | 523,53 | 2094,14 | 2,094 | 0,0000020941367902 | 0,0000000577756660 | 5,778 |
| 75 | 22.0 | 0,621 | 1,610 | 483,81 | 1935,25 | 1,935 | 0,0000019352506751 | 0,0000000533921171 | 5,339 |
| 76 | 24.0 | 0,499 | 2,004 | 949,72 | 3798,90 | 3,799 | 0,0000037988979225 | 0,0000000104808749 | 10,481 |
| 77 | 26.0 | 0,442 | 2,262 | 1269,52 | 5078,07 | 5,078 | 0,0000050780681313 | 0,0000000140100974 | 14,010 |
| 78 | 27.0 | 0,554 | 1,805 | 711,07 | 2844,29 | 2,844 | 0,0000028442855557 | 0,0000000784717088 | 7,847 |
| 79 | 28.0 | 0,370 | 2,703 | 1839,78 | 7359,10 | 7,359 | 0,0000073591018262 | 0,0000000203032109 | 20,303 |
| 80 | 30.0 | 0,539 | 1,855 | 770,71 | 3082,84 | 3,083 | 0,0000030828388089 | 0,0000000850532144 | 8,505 |
| 81 | 31.0 | 0,916 | | OUT OF STANDARDS | | | | | |
| 82 | 32.0 | 0,526 | 1,901 | 825,51 | 3302,05 | 3,302 | 0,0000033020499125 | 0,0000000911010846 | 9,110 |
| 83 | 33.0 | 0,599 | 1,669 | 552,16 | 2208,66 | 2,209 | 0,0000022086590104 | 0,0000000609352483 | 6,094 |
| 84 | 34.0 | 0,595 | 1,681 | 565,20 | 2260,80 | 2,261 | 0,0000022608034461 | 0,0000000623738743 | 6,237 |
| 85 | 35.0 | 0,582 | 1,718 | 608,96 | 2435,83 | 2,436 | 0,0000024358294021 | 0,0000000672027093 | 6,720 |
| 86 | 26.0 | 0,894 | | OUT OF STANDARDS | | | | | |
| 87 | 37.0 | 0,808 | 1,238 | 66,43 | 265,72 | 0,266 | 0,0000002657155279 | 0,0000000073308925 | 0,733 |
| 88 | 38.0 | 0,759 | 1,318 | 153,97 | 615,90 | 0,616 | 0,0000006158979546 | 0,0000000169921634 | 1,699 |
| 89 | 41.0 | 0,746 | 1,340 | 179,33 | 717,31 | 0,717 | 0,0000007173071495 | 0,0000000197899672 | 1,979 |
| 90 | 43.0 | 0,717 | 1,395 | 239,54 | 958,17 | 0,958 | 0,0000009581651473 | 0,0000000264350590 | 2,644 |
| 91 | 44.0 | 0,710 | 1,408 | 254,89 | 1019,56 | 1,020 | 0,0000010195607221 | 0,0000000281289169 | 2,813 |
| 92 | 46.0 | 0,782 | 1,279 | 111,38 | 445,53 | 0,446 | 0,0000004455333861 | 0,0000000122919325 | 1,229 |
| 93 | 48.0 | 0,647 | 1,546 | 409,69 | 1638,76 | 1,639 | 0,0000016387583506 | 0,0000000452121158 | 4,521 |
| 94 | 49.0 | 0,611 | 1,637 | 514,20 | 2056,80 | 2,057 | 0,0000020568001629 | 0,0000000567455764 | 5,675 |
| 95 | 50.0 | 0,729 | 1,372 | 213,98 | 855,94 | 0,856 | 0,0000008559378851 | 0,0000000236146853 | 2,361 |
| 96 | 52.0 | 0,641 | 1,560 | 426,20 | 1704,80 | 1,705 | 0,0000017048040420 | 0,0000000470342670 | 4,703 |

| Reading Nº | Samples | Absorvance(nm) | 1/Abs(nm) | Concentration (pg/mL) | Concentration (pg/mL) | Concentration (ng/mL) | Concentration (g/L) | Concentration (mol/L) | Concentration (nmol/L) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 1.1 | 0,383 | 2,611 | 486,775 | 1947,10 | 1,947 | 0,0000019470994160 | 0,0000000053719015 | 5,372 |
| 11 | 1.2 | 0,394 | 2,538 | 450,068 | 1800,27 | 1,800 | 0,0000018002732727 | 0,0000000049668192 | 4,967 |
| 12 | 1.3 | 0,421 | 2,375 | 373,596 | 1494,39 | 1,494 | 0,0000014943858507 | 0,0000000041228987 | 4,123 |
| 13 | 2.1 | 0,537 | 1,862 | 182,204 | 728,82 | 0,729 | 0,0000007288176435 | 0,0000000020107533 | 2,011 |
| 14 | 2.2 | 0,789 | 1,267 | OUT OF STANDARDS | | | | | |
| 15 | 2.3 | 0,426 | 2,347 | 361,260 | 1445,04 | 1,445 | 0,0000014450398960 | 0,0000000039867569 | 3,987 |
| 16 | 3.1 | 0,416 | 2,404 | 386,460 | 1545,84 | 1,546 | 0,0000015458408432 | 0,0000000042648591 | 4,265 |
| 17 | 3.2 | 0,468 | 2,137 | 275,237 | 1100,95 | 1,101 | 0,0000011009486624 | 0,0000000030374349 | 3,037 |
| 18 | 3.3 | 0,468 | 2,137 | 275,237 | 1100,95 | 1,101 | 0,0000011009486624 | 0,0000000030374349 | 3,037 |
| 19 | 7.1 | 0,422 | 2,370 | 371,088 | 1484,35 | 1,484 | 0,0000014843513704 | 0,0000000040952143 | 4,095 |
| 20 | 7.2 | 0,402 | 2,488 | 425,527 | 1702,11 | 1,702 | 0,0000017021080706 | 0,0000000046959887 | 4,696 |
| 21 | 7.3 | 0,677 | 1,477 | OUT OF STANDARDS | | | | | |
| 22 | 9.1 | 0,390 | 2,564 | 463,002 | 1852,01 | 1,852 | 0,0000018520074688 | 0,0000000051095499 | 5,110 |
| 23 | 9.2 | 0,473 | 2,114 | 266,768 | 1067,07 | 1,067 | 0,0000010670737930 | 0,0000000029439767 | 2,944 |
| 24 | 9.3 | 0,502 | 1,992 | 223,508 | 894,03 | 0,894 | 0,0000008940315893 | 0,0000000024665662 | 2,467 |
| 25 | 10.1 | 0,331 | 3,021 | 721,700 | 2886,80 | 2,887 | 0,0000028868018160 | 0,0000000079644701 | 7,964 |
| 26 | 10.2 | 0,355 | 2,817 | 598,698 | 2394,79 | 2,395 | 0,0000023947904305 | 0,0000000066070475 | 6,607 |
| 27 | 10.3 | 0,419 | 2,387 | 378,677 | 1514,71 | 1,515 | 0,0000015147086982 | 0,0000000041789679 | 4,179 |
| 28 | 11.1 | 0,384 | 2,604 | 483,287 | 1933,15 | 1,933 | 0,0000019331465451 | 0,0000000053334066 | 5,333 |
| 29 | 11.2 | 0,296 | 3,378 | 965,559 | 3862,24 | 3,862 | 0,0000038622352374 | 0,0000000106556178 | 10,656 |
| 30 | 11.3 | 0,425 | 2,353 | 363,686 | 1454,75 | 1,455 | 0,0000014547454671 | 0,0000000040135338 | 4,014 |
| 31 | 12.1 | 0,350 | 2,857 | 621,987 | 2487,95 | 2,488 | 0,0000024879477551 | 0,0000000068640616 | 6,864 |
| 32 | 12.2 | 0,441 | 2,268 | 327,161 | 1308,64 | 1,309 | 0,0000013086428029 | 0,0000000036104475 | 3,610 |
| 33 | 12.3 | 0,488 | 2,049 | 243,219 | 972,87 | 0,973 | 0,0000009728740554 | 0,0000000026840867 | 2,684 |
| 34 | 13.1 | 0,486 | 2,058 | 246,206 | 984,82 | 0,985 | 0,0000009848248715 | 0,0000000027170581 | 2,717 |
| 35 | 13.2 | 0,657 | 1,522 | 96,893 | 387,57 | 0,388 | 0,0000003875733752 | 0,0000000010692859 | 1,069 |
| 36 | 13.3 | 0,425 | 2,353 | 363,686 | 1454,75 | 1,455 | 0,0000014547454671 | 0,0000000040135338 | 4,014 |
| 37 | 14.1 | 0,701 | 1,427 | OUT OF STANDARDS | | | | | |
| 38 | 14.2 | 0,520 | 1,923 | 200,970 | 803,88 | 0,804 | 0,0000008038818935 | 0,0000000022178500 | 2,218 |
| 39 | 14.3 | 0,464 | 2,155 | 282,252 | 1129,01 | 1,129 | 0,0000011290069084 | 0,0000000031148455 | 3,115 |
| 40 | 25.1 | 0,543 | 1,842 | 176,102 | 704,41 | 0,704 | 0,0000007044067025 | 0,0000000019434053 | 1,943 |
| 41 | 25.2 | 0,328 | 3,049 | 739,259 | 2957,04 | 2,957 | 0,0000029570362641 | 0,0000000081582416 | 8,158 |
| 42 | 25.3 | 0,410 | 2,439 | 402,632 | 1610,53 | 1,611 | 0,0000016105276383 | 0,0000000044433252 | 4,443 |
| 43 | 54.1 | 0,368 | 2,717 | 543,099 | 2172,39 | 2,172 | 0,0000021723948771 | 0,0000000059934748 | 5,993 |
| 44 | 54.2 | 0,453 | 2,208 | 302,711 | 1210,84 | 1,211 | 0,0000012108431377 | 0,0000000033406256 | 3,341 |
| 45 | 54.3 | 0,487 | 2,053 | 244,707 | 978,83 | 0,979 | 0,0000009788270096 | 0,0000000027005104 | 2,701 |
| 46 | 55.1 | 0,413 | 2,421 | 394,443 | 1577,77 | 1,578 | 0,0000015777720866 | 0,0000000043529550 | 4,353 |
| 47 | 55.2 | 0,434 | 2,304 | 342,554 | 1370,21 | 1,370 | 0,0000013702149173 | 0,0000000037803204 | 3,780 |
| 48 | 55.3 | 0,337 | 2,967 | 688,148 | 2752,59 | 2,753 | 0,0000027525931111 | 0,0000000075941983 | 7,594 |
| 49 | 56.1 | 0,507 | 1,972 | 216,948 | 867,79 | 0,868 | 0,0000008677930038 | 0,0000000023941759 | 2,394 |
| 50 | 56.2 | 0,440 | 2,273 | 329,307 | 1317,23 | 1,317 | 0,0000013172261157 | 0,0000000036341282 | 3,634 |
| 51 | 56.3 | 0,461 | 2,169 | 287,658 | 1150,63 | 1,151 | 0,0000011506307958 | 0,0000000031745042 | 3,175 |
| 52 | 57.1 | 0,302 | 3,311 | 916,952 | 3667,81 | 3,668 | 0,0000036678063313 | 0,0000000101192030 | 10,119 |
| 53 | 57.2 | 0,290 | 3,448 | 1017,549 | 4070,20 | 4,070 | 0,0000040701956718 | 0,0000000112293651 | 11,229 |
| 54 | 57.3 | 0,335 | 2,985 | 699,107 | 2796,43 | 2,796 | 0,0000027964291022 | 0,0000000077151385 | 7,715 |
| 55 | 58.1 | 0,354 | 2,825 | 603,266 | 2413,06 | 2,413 | 0,0000024130642102 | 0,0000000066574635 | 6,657 |
| 56 | 58.2 | 0,376 | 2,660 | 512,098 | 2048,39 | 2,048 | 0,0000020483904210 | 0,0000000056513558 | 5,651 |
| 57 | 58.3 | 0,467 | 2,141 | 276,970 | 1107,88 | 1,108 | 0,0000011078818750 | 0,0000000030565631 | 3,057 |
| 58 | 59.1 | 0,408 | 2,451 | 408,209 | 1632,84 | 1,633 | 0,0000016328355094 | 0,0000000045048709 | 4,505 |
| 59 | 59.2 | 0,465 | 2,151 | 280,478 | 1121,91 | 1,122 | 0,0000011219104914 | 0,0000000030952670 | 3,095 |
| 60 | 59.3 | 0,450 | 2,222 | 308,605 | 1234,42 | 1,234 | 0,0000012344217284 | 0,0000000034056771 | 3,406 |
| 61 | 60.1 | 0,466 | 2,146 | 278,717 | 1114,87 | 1,115 | 0,0000011148689834 | 0,0000000030758400 | 3,076 |
| 62 | 60.2 | 0,455 | 2,198 | 298,858 | 1195,43 | 1,195 | 0,0000011954325516 | 0,0000000032981089 | 3,298 |
| 63 | 60.3 | 0,231 | 4,329 | 1792,541 | 7170,16 | 7,170 | 0,0000071701636356 | 0,0000000197819446 | 19,782 |
| 64 | 61.1 | 0,448 | 2,232 | 312,614 | 1250,46 | 1,250 | 0,0000012504562372 | 0,0000000034499151 | 3,450 |
| 65 | 61.2 | 0,391 | 2,558 | 459,725 | 1838,90 | 1,839 | 0,0000018389015310 | 0,0000000050733916 | 5,073 |
| 66 | 61.3 | 0,540 | 1,852 | 179,121 | 716,48 | 0,716 | 0,0000007164837311 | 0,0000000019767250 | 1,977 |
| 67 | 62.1 | 0,348 | 2,874 | 631,623 | 2526,49 | 2,526 | 0,0000025264932012 | 0,0000000069704056 | 6,970 |
| 68 | 62.2 | 0,338 | 2,959 | 682,751 | 2731,00 | 2,731 | 0,0000027310028977 | 0,0000000075346325 | 7,535 |
| 69 | 62.3 | 0,581 | 1,721 | 142,811 | 571,24 | 0,571 | 0,0000005712430457 | 0,0000000015760168 | 1,576 |
| 70 | 64.1 | 0,499 | 2,004 | 227,561 | 910,24 | 0,910 | 0,0000009102440720 | 0,0000000025112952 | 2,511 |
| 71 | 64.2 | 0,491 | 2,037 | 238,820 | 955,28 | 0,955 | 0,0000009552806607 | 0,0000000026355478 | 2,636 |
| 72 | 64.3 | 0,458 | 2,183 | 293,192 | 1172,77 | 1,173 | 0,0000011727677687 | 0,0000000032355785 | 3,236 |
| 73 | 65.1 | 0,622 | 1,608 | 115,254 | 461,02 | 0,461 | 0,0000004610163187 | 0,0000000012719095 | 1,272 |
| 74 | 65.2 | 0,402 | 2,488 | 425,527 | 1702,11 | 1,702 | 0,0000017021080706 | 0,0000000046959887 | 4,696 |
| 75 | 65.3 | 0,584 | 1,712 | 140,531 | 562,12 | 0,562 | 0,0000005621230325 | 0,0000000015508554 | 1,551 |
| 76 | 1.0 | 0,368 | 2,717 | 543,099 | 2172,39 | 2,172 | 0,0000021723948771 | 0,0000000059934748 | 5,993 |
| 77 | 2.0 | 0,391 | 2,558 | 459,725 | 1838,90 | 1,839 | 0,0000018389015310 | 0,0000000050733916 | 5,073 |
| 78 | 3.0 | 0,319 | 3,135 | 795,316 | 3181,27 | 3,181 | 0,0000031812655709 | 0,0000000087768735 | 8,777 |
| 79 | 7.0 | 0,388 | 2,577 | 469,643 | 1878,57 | 1,879 | 0,0000018785717334 | 0,0000000051828388 | 5,183 |
| 80 | 9.0 | 0,552 | 1,812 | 167,414 | 669,66 | 0,670 | 0,0000006696556942 | 0,0000000018475299 | 1,848 |
| 81 | 10.0 | 0,420 | 2,381 | 376,126 | 1504,50 | 1,505 | 0,0000015045046712 | 0,0000000041508157 | 4,151 |
| 82 | 11.0 | 0,596 | 1,678 | 131,854 | 527,42 | 0,527 | 0,0000005274166101 | 0,0000000014551029 | 1,455 |
| 83 | 13.0 | 0,541 | 1,848 | 178,107 | 712,43 | 0,712 | 0,0000007124298228 | 0,0000000019655405 | 1,966 |
| 84 | 14.0 | 0,601 | 1,664 | 128,438 | 513,75 | 0,514 | 0,0000005137519447 | 0,0000000014174031 | 1,417 |
| 85 | 15.0 | 0,502 | 1,992 | 223,508 | 894,03 | 0,894 | 0,0000008940315893 | 0,0000000024665662 | 2,467 |
| 86 | 16.0 | 0,401 | 2,494 | 428,502 | 1714,01 | 1,714 | 0,0000017140070781 | 0,0000000047288172 | 4,729 |
| 87 | 25.0 | 0,408 | 2,451 | 408,209 | 1632,84 | 1,633 | 0,0000016328355094 | 0,0000000045048709 | 4,505 |
| 88 | 54.0 | 0,532 | 1,880 | 187,491 | 749,96 | 0,750 | 0,0000007499624761 | 0,0000000020690903 | 2,069 |
| 89 | 55.0 | 0,397 | 2,519 | 440,663 | 1762,65 | 1,763 | 0,0000017626534870 | 0,0000000048630290 | 4,863 |
| 90 | 56.0 | 0,597 | 1,675 | 131,162 | 524,65 | 0,525 | 0,0000005246474175 | 0,0000000014474629 | 1,447 |
| 91 | 57.0 | 0,561 | 1,783 | 159,251 | 637,00 | 0,637 | 0,0000006370034391 | 0,0000000017574448 | 1,757 |
| 92 | 59.0 | 0,398 | 2,513 | 437,583 | 1750,33 | 1,750 | 0,0000017503320664 | 0,0000000048290351 | 4,829 |
| 93 | 61.0 | 0,335 | 2,985 | 699,107 | 2796,43 | 2,796 | 0,0000027964291022 | 0,0000000077151385 | 7,715 |
| 94 | 62.0 | 0,734 | 1,362 | OUT OF STANDARDS | | | | | |
| 95 | 64.0 | 0,603 | 1,658 | 127,103 | 508,41 | 0,508 | 0,0000005084111049 | 0,0000000014026682 | 1,403 |
| 96 | 65.0 | 0,413 | 2,421 | 394,443 | 1577,77 | 1,578 | 0,0000015777720866 | 0,0000000043529550 | 4,353 |



APPENDIX 12: Cortisol statistic results to Cross-sectional study.

**Pairwise Comparisons**

| Sample 1-Sample 2 | Test Statistic | Std. Error | Std. Test Statistic | Sig. | Adj. Sig.[a] |
|---|---|---|---|---|---|
| Cortisol3-Cortsol2 | 0,596 | 0,206 | 2,888 | 0,004 | 0,012 |
| Cortisol3-Cortisol1 | 0,681 | 0,206 | 3,301 | 0,001 | 0,003 |
| Cortsol2-Cortisol1 | 0,085 | 0,206 | 0,413 | 0,680 | 1,000 |

Each row tests the null hypothesis that the Sample 1 and Sample 2

a. Significance values have been adjusted by the Bonferroni correction for

Poisson Regression

**Parameter Estimates**

| Parameter | B | Std. Error | Interval | | Hypothesis Test | | |
|---|---|---|---|---|---|---|---|
| | | | Lower | Upper | Wald Chi-Square | df | Sig. |
| (Intercept) | 4,059 | 0,9795 | 2,139 | 5,978 | 17,170 | 1 | 0,000 |
| Sbp_mmHg_T2 | 0,007 | 0,0025 | 0,002 | 0,012 | 8,533 | 1 | 0,003 |
| Dbp_mmHg_T2 | -0,005 | 0,0044 | -0,014 | 0,003 | 1,527 | 1 | 0,217 |
| Beats_min_T2 | -0,003 | 0,0046 | -0,012 | 0,006 | 0,521 | 1 | 0,470 |
| Breaths_min_T2 | 0,091 | 0,0337 | 0,025 | 0,158 | 7,352 | 1 | 0,007 |
| Percent_SpO2_T2 | -0,034 | 0,0070 | -0,047 | -0,020 | 22,910 | 1 | 0,000 |
| VAS_T2 | -0,019 | 0,0163 | -0,051 | 0,013 | 1,400 | 1 | 0,237 |
| Cortsol2 | -0,021 | 0,0188 | -0,058 | 0,015 | 1,297 | 1 | 0,255 |
| (Scale) | 1[a] | | | | | | |

Dependent Variable: Movimentos_Global

a. Fixed at the displayed value.

| | | | STAI_S_T1 | VAS_T2 |
|---|---|---|---|---|
| | CortisolT2 | Correlation Coefficient | -0,187 | 0,019 |
| | | Sig. (2-tailed) | 0,198 | 0,895 |
| | | N | 49 | 49 |

APPENDIX 13: Physiological results for Cross-sectional study.

| ID | T1 | | | | | T2 | | | | | T3 | | | | | T4 | | | | | T5 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | bp (mmHg | bp (mmHg | Beats/min | breaths/mi | %SpO2 | bp (mmHg | bp (mmHg | Beats/min | breaths/mi | %SpO2 | Sbp (mm | Dbp (mm | Beats/mir | Breaths/m | %SpO2 | Sbp (mm | Dbp (mm | Beats/min | Breaths/m | %SpO2 | Sbp (mmH | Dbp (mmH | Beats/min | Breaths/m | %SpO2 |
| 02 | 129 | 79 | 55 | 15 | 97 | 137 | 70 | 59 | 16 | 99 | 143 | 78 | 63 | 16 | 99 | 131 | 76 | 70 | 17 | 96 | 127 | 83 | 60 | 16 | 96 |
| 03 | 169 | 89 | 70 | 14 | 99 | 158 | 63 | 89 | 14 | 99 | 136 | 73 | 66 | 14 | 99 | 163 | 87 | 71 | 16 | 99 | 157 | 81 | 68 | 15 | 81 |
| 04 | 158 | 89 | 64 | 13 | 70 | 169 | 69 | 62 | 14 | 89 | 137 | 73 | 62 | 13 | 99 | 166 | 78 | 69 | 14 | 94 | 149 | 83 | 64 | 14 | 99 |
| 05 | 158 | 78 | 57 | 14 | 97 | 149 | 104 | 53 | 14 | 96 | 139 | 68 | 57 | 14 | 94 | 159 | 76 | 61 | 15 | 92 | 171 | 92 | 61 | 15 | 97 |
| 06 | 140 | 95 | 78 | 13 | 99 | 138 | 99 | 84 | 13 | 98 | 136 | 90 | 79 | 15 | 99 | 137 | 91 | 83 | 16 | 98 | 140 | 89 | 70 | 15 | 99 |
| 07 | 182 | 109 | 65 | 12 | 99 | 178 | 116 | 60 | 12 | 98 | 153 | 95 | 64 | 12 | 99 | 169 | 102 | 59 | 14 | 97 | 187 | 105 | 70 | 13 | 99 |
| 08 | 118 | 71 | 72 | 13 | 74 | 108 | 67 | 69 | 14 | 97 | 133 | 76 | 76 | 14 | 99 | 120 | 74 | 82 | 15 | 96 | 124 | 73 | 88 | 14 | 98 |
| 09 | 123 | 72 | 71 | 16 | 99 | 126 | 74 | 79 | 17 | 98 | 123 | 75 | 77 | 17 | 98 | 108 | 61 | 50 | 18 | 99 | 119 | 74 | 80 | 17 | 99 |
| 10 | 140 | 89 | 75 | 15 | 99 | 132 | 88 | 73 | 14 | 70 | 125 | 85 | 78 | 14 | 98 | 152 | 127 | 92 | 15 | 97 | 126 | 81 | 74 | 16 | 99 |
| 11 | 155 | 83 | 66 | 15 | 99 | 157 | 84 | 65 | 15 | 99 | 157 | 77 | 65 | 16 | 99 | 160 | 85 | 68 | 17 | 72 | 148 | 81 | 64 | 18 | 99 |
| 01 | 149 | 67 | 61 | 13 | 95 | 158 | 68 | 64 | 14 | 93 | 151 | 64 | 62 | 13 | 97 | 152 | 55 | 56 | 15 | 97 | 163 | 68 | 63 | 15 | 97 |
| 12 | 138 | 88 | 70 | 14 | 99 | 136 | 89 | 70 | 14 | 99 | 138 | 80 | 73 | 15 | 99 | 135 | 82 | 84 | 16 | 98 | 129 | 87 | 46 | 14 | 99 |
| 13 | 153 | 73 | 70 | 13 | 97 | 150 | 87 | 71 | 13 | 97 | 147 | 73 | 77 | 12 | 96 | 147 | 76 | 80 | 14 | 97 | 157 | 80 | 70 | 13 | 97 |
| 14 | 186 | 113 | 71 | 12 | 93 | 150 | 105 | 70 | 12 | 98 | 151 | 99 | 73 | 12 | 99 | 183 | 105 | 49 | 13 | 93 | 203 | 119 | 75 | 13 | 99 |
| 15 | 179 | 86 | 74 | 11 | 95 | 191 | 87 | 67 | 12 | 97 | 181 | 71 | 76 | 12 | 96 | 198 | 74 | 70 | 14 | 94 | 197 | 76 | 74 | 12 | 97 |
| 17 | 140 | 78 | 72 | 14 | 96 | 141 | 82 | 74 | 15 | 95 | 138 | 77 | 71 | 18 | 93 | 156 | 81 | 77 | 20 | 96 | 134 | 78 | 84 | 15 | 94 |
| 18 | 171 | 96 | 101 | 16 | 98 | 159 | 89 | 95 | 16 | 96 | 150 | 76 | 101 | 16 | 99 | 142 | 72 | 120 | 19 | 96 | 167 | 86 | 98 | 15 | 98 |
| 19 | 98 | 52 | 50 | 14 | 99 | 98 | 56 | 55 | 15 | 98 | 113 | 58 | 54 | 13 | 99 | 123 | 57 | 72 | 16 | 94 | 104 | 62 | 56 | 14 | 99 |
| 20 | 122 | 86 | 70 | 18 | 98 | 112 | 76 | 61 | 17 | 99 | 116 | 72 | 65 | 17 | 97 | 112 | 74 | 63 | 20 | 78 | 122 | 78 | 74 | 16 | 97 |
| 21 | 191 | 86 | 86 | 14 | 99 | 180 | 80 | 80 | 15 | 97 | 197 | 75 | 82 | 14 | 97 | 209 | 89 | 96 | 18 | 96 | 193 | 84 | 90 | 13 | 98 |
| 22 | 163 | 70 | 79 | 18 | 97 | 161 | 69 | 74 | 16 | 98 | 154 | 64 | 77 | 14 | 97 | 162 | 59 | 73 | 16 | 96 | 167 | 75 | 82 | 15 | 91 |
| 23 | 182 | 78 | 55 | 16 | 97 | 194 | 89 | 55 | 15 | 97 | 160 | 81 | 44 | 16 | 96 | 192 | 78 | 65 | 16 | 98 | 192 | 92 | 57 | 18 | 96 |
| 26 | 135 | 80 | 64 | 14 | 98 | 137 | 83 | 64 | 13 | 97 | 145 | 79 | 62 | 14 | 96 | 119 | 99 | 59 | 18 | 96 | 158 | 88 | 75 | 15 | 99 |
| 27 | 158 | 88 | 83 | 14 | 98 | 156 | 89 | 76 | 14 | 96 | 170 | 87 | 95 | 17 | 96 | 163 | 88 | 103 | 18 | 97 | 136 | 82 | 92 | 15 | 93 |
| 28 | 128 | 75 | 73 | 14 | 97 | 147 | 75 | 81 | 15 | 97 | 127 | 95 | 90 | 14 | 98 | 144 | 68 | 91 | 17 | 96 | 155 | 73 | 89 | 15 | 99 |
| 29 | 184 | 114 | 72 | 15 | 97 | 175 | 108 | 63 | 15 | 72 | 154 | 105 | 67 | 16 | 99 | 175 | 91 | 86 | 18 | 97 | 159 | 92 | 74 | 14 | 98 |
| 30 | 165 | 92 | 98 | 16 | 99 | 147 | 93 | 77 | 15 | 99 | 146 | 90 | 89 | 15 | 99 | 121 | 99 | 94 | 16 | 99 | 157 | 100 | 87 | 17 | 99 |
| 31 | 166 | 78 | 58 | 15 | 98 | 178 | 89 | 45 | 15 | 98 | 183 | 74 | 42 | 13 | 99 | 180 | 88 | 59 | 14 | 96 | 191 | 86 | 49 | 14 | 99 |
| 16 | 145 | 117 | 61 | 17 | 97 | 154 | 67 | 65 | 16 | 96 | 141 | 69 | 68 | 13 | 99 | 133 | 64 | 76 | 17 | 69 | 146 | 66 | 70 | 13 | 92 |
| 24 | 144 | 67 | 51 | 16 | 98 | 140 | 73 | 53 | 15 | 94 | 127 | 69 | 57 | 13 | 97 | 162 | 80 | 53 | 12 | 99 | 156 | 78 | 50 | 12 | 99 |
| 25 | 149 | 87 | 74 | 16 | 97 | 144 | 84 | 86 | 14 | 96 | 157 | 69 | 79 | 12 | 98 | 158 | 81 | 106 | 15 | 98 | 145 | 87 | 97 | 12 | 98 |
| 34 | 137 | 82 | 66 | 12 | 97 | 139 | 86 | 59 | 12 | 97 | 141 | 73 | 75 | 13 | 98 | 149 | 75 | 69 | 12 | 96 | 167 | 107 | 70 | 14 | 99 |
| 35 | 180 | 86 | 72 | 13 | 99 | 179 | 86 | 72 | 12 | 98 | 164 | 74 | 71 | 13 | 97 | 175 | 85 | 68 | 15 | 92 | 196 | 82 | 71 | 13 | 99 |
| 36 | 131 | 77 | 76 | 14 | 98 | 127 | 79 | 71 | 13 | 98 | 115 | 69 | 78 | 14 | 99 | 125 | 73 | 92 | 13 | 98 | 133 | 76 | 83 | 12 | 99 |
| 37 | 154 | 84 | 59 | 13 | 97 | 156 | 86 | 60 | 12 | 96 | 172 | 88 | 57 | 13 | 95 | 158 | 78 | 95 | 17 | 98 | 151 | 83 | 68 | 11 | 93 |
| 38 | 163 | 79 | 53 | 13 | 96 | 188 | 79 | 51 | 12 | 94 | 186 | 76 | 51 | 12 | 97 | 195 | 83 | 56 | 12 | 99 | 215 | 79 | 53 | 11 | 98 |
| 40 | 164 | 78 | 44 | 16 | 99 | 175 | 86 | 49 | 18 | 98 | 180 | 89 | 54 | 18 | 99 | 195 | 90 | 65 | 19 | 96 | 179 | 96 | 64 | 18 | 97 |
| 32 | 131 | 68 | 77 | 14 | 95 | 123 | 70 | 78 | 13 | 97 | 107 | 62 | 34 | 12 | 96 | 111 | 66 | 83 | 12 | 92 | 125 | 69 | 83 | 14 | 97 |
| 33 | 154 | 97 | 89 | 13 | 96 | 126 | 80 | 87 | 14 | 96 | 128 | 79 | 73 | 12 | 97 | 143 | 86 | 112 | 13 | 97 | 141 | 84 | 92 | 12 | 97 |
| 39 | 143 | 78 | 70 | 13 | 98 | 140 | 70 | 68 | 13 | 98 | 139 | 63 | 64 | 12 | 98 | 160 | 81 | 70 | 15 | 98 | 154 | 76 | 65 | 13 | 99 |
| 45 | 159 | 97 | 66 | 14 | 97 | 177 | 102 | 66 | 12 | 98 | 169 | 92 | 73 | 12 | 98 | 166 | 88 | 80 | 18 | 97 | 165 | 93 | 69 | 12 | 97 |
| 46 | 160 | 84 | 62 | 13 | 93 | 166 | 78 | 47 | 12 | 80 | 166 | 82 | 73 | 12 | 93 | 140 | 69 | 65 | 17 | 91 | 151 | 63 | 66 | 13 | 97 |
| 42 | 137 | 88 | 62 | 13 | 97 | 146 | 85 | 63 | 12 | 96 | 140 | 84 | 58 | 14 | 94 | 143 | 84 | 69 | 18 | 95 | 151 | 89 | 60 | 12 | 97 |
| 43 | 152 | 88 | 54 | 12 | 97 | 159 | 93 | 55 | 11 | 98 | 181 | 90 | 52 | 12 | 99 | 156 | 90 | 54 | 16 | 97 | 191 | 102 | 51 | 12 | 99 |
| 41 | 136 | 80 | 69 | 12 | 99 | 160 | 82 | 67 | 11 | 99 | 140 | 78 | 35 | 12 | 99 | 144 | 106 | 67 | 14 | 97 | 135 | 72 | 67 | 13 | 99 |
| 44 | 151 | 86 | 59 | 12 | 99 | 163 | 87 | 63 | 13 | 90 | 163 | 82 | 55 | 13 | 92 | 150 | 82 | 50 | 15 | 90 | 155 | 92 | 85 | 14 | 97 |
| 49 | 147 | 87 | 85 | 12 | 99 | 146 | 83 | 76 | 13 | 99 | 143 | 80 | 69 | 14 | 99 | 166 | 83 | 76 | 16 | 99 | 149 | 74 | 80 | 13 | 97 |
| 50 | 159 | 84 | 71 | 12 | 98 | 156 | 82 | 67 | 14 | 97 | 161 | 80 | 63 | 14 | 99 | 143 | 84 | 59 | 14 | 99 | 142 | 71 | 58 | 12 | 97 |
| 51 | 167 | 103 | 67 | 14 | 97 | 177 | 106 | 63 | 14 | 98 | 164 | 89 | 73 | 13 | 99 | 164 | 105 | 71 | 15 | 97 | 173 | 82 | 66 | 13 | 98 |
| 52 | 177 | 87 | 60 | 12 | 98 | 169 | 92 | 67 | 14 | 99 | 136 | 68 | 70 | 12 | 97 | 180 | 85 | 84 | 14 | 99 | 179 | 92 | 65 | 12 | 99 |
| 53 | 127 | 76 | 55 | 11 | 98 | 122 | 69 | 55 | 13 | 97 | 125 | 74 | 56 | 11 | 97 | 139 | 69 | 64 | 14 | 97 | 134 | 80 | 61 | 13 | 99 |
| 54 | 138 | 82 | 91 | 13 | 98 | 143 | 81 | 88 | 12 | 97 | 154 | 78 | 89 | 14 | 98 | 152 | 83 | 91 | 16 | 96 | 170 | 87 | 72 | 13 | 97 |
| 48 | 174 | 115 | 96 | 16 | 86 | 170 | 110 | 80 | 15 | 90 | 172 | 101 | 95 | 15 | 85 | 164 | 90 | 100 | 17 | 97 | 157 | 102 | 84 | 14 | 90 |
| 55 | 176 | 84 | 65 | 13 | 97 | 176 | 76 | 62 | 12 | 95 | 169 | 69 | 63 | 14 | 97 | 180 | 72 | 83 | 12 | 98 | 181 | 80 | 65 | 12 | 98 |
| 56 | 168 | 87 | 64 | 12 | 97 | 157 | 81 | 61 | 12 | 98 | 156 | 65 | 56 | 12 | 97 | 153 | 89 | 61 | 11 | 89 | 161 | 68 | 69 | 12 | 97 |
| 57 | 146 | 94 | 62 | 11 | 99 | 143 | 91 | 72 | 13 | 97 | 153 | 89 | 61 | 11 | 89 | 149 | 88 | 65 | 13 | 95 | 149 | 90 | 56 | 11 | 99 |
| 58 | 150 | 81 | 71 | 11 | 97 | 150 | 68 | 59 | 12 | 99 | 164 | 67 | 62 | 11 | 98 | 141 | 72 | 63 | 14 | 99 | 160 | 69 | 63 | 12 | 98 |
| 59 | 163 | 102 | 70 | 12 | 98 | 150 | 87 | 68 | 13 | 99 | 144 | 88 | 67 | 12 | 97 | 158 | 84 | 68 | 14 | 99 | 156 | 81 | 75 | 12 | 99 |
| 64 | 190 | 95 | 59 | 14 | 99 | 187 | 92 | 60 | 12 | 98 | 174 | 88 | 65 | 15 | 99 | 180 | 102 | 87 | 15 | 99 | 167 | 93 | 56 | 13 | 97 |
| 60 | 177 | 86 | 55 | 12 | 96 | 178 | 89 | 55 | 12 | 97 | 165 | 77 | 66 | 12 | 97 | 185 | 90 | 62 | 13 | 97 | 192 | 83 | 69 | 12 | 93 |
| 61 | 152 | 77 | 42 | 12 | 99 | 169 | 85 | 71 | 12 | 99 | 150 | 62 | 79 | 12 | 95 | 157 | 75 | 99 | 15 | 98 | 151 | 84 | 69 | 12 | 85 |
| 62 | 136 | 79 | 65 | 12 | 98 | 139 | 75 | 63 | 13 | 98 | 135 | 68 | 77 | 12 | 97 | 132 | 73 | 80 | 14 | 94 | 129 | 64 | 72 | 11 | 99 |
| 65 | 103 | 73 | 65 | 11 | 99 | 118 | 74 | 78 | 12 | 98 | 106 | 69 | 61 | 13 | 99 | 105 | 68 | 50 | 14 | 90 | 131 | 82 | 61 | 11 | 91 |

APPENDIX 14: Physiological statistic results.

| | Minimum | Maximum | Mean | Standard Deviation |
|---|---|---|---|---|
| Sbp_mmHg_T1 | 98 | 191 | 153 | 21 |
| Dbp_mmHg_T1 | 52 | 117 | 85 | 12 |
| Beats_minT1 | 12 | 101 | 67 | 14 |
| Breaths_min_T1 | 11 | 59 | 14 | 6 |
| Percent_SpO2_T1 | 70 | 99 | 97 | 5 |
| Sbp_mmHg_T2 | 98 | 194 | 153 | 21 |
| Dbp_mmHg_T2 | 56 | 116 | 84 | 12 |
| Beats_min_T2 | 45 | 95 | 67 | 11 |
| Breaths_min_T2 | 11 | 18 | 14 | 2 |
| Percent_SpO2_T2 | 70 | 99 | 96 | 5 |
| Sbp_mmHg_T3 | 106 | 197 | 149 | 20 |
| Dbp_mmHg_T3 | 58 | 105 | 78 | 10 |
| Beats_min_T3 | 34 | 101 | 68 | 13 |
| Breaths_min_T3 | 11 | 18 | 14 | 2 |
| Percent_SpO2_T3 | 85 | 99 | 97 | 3 |
| Sbp_mmHg_T4 | 105 | 209 | 154 | 23 |
| Dbp_mmHg_T4 | 55 | 127 | 82 | 13 |
| Beats_min_T4 | 19 | 120 | 74 | 18 |
| Breaths_min_T4 | 11 | 65 | 16 | 7 |
| Percent_SpO2_T4 | 69 | 99 | 95 | 6 |
| Sbp_mmHg_T5 | 104 | 215 | 157 | 23 |
| Dbp_mmHg_T5 | 62 | 119 | 83 | 11 |
| Beats_min_T5 | 46 | 98 | 70 | 12 |
| Breaths_min_T5 | 11 | 18 | 14 | 2 |
| Percent_SpO2_T5 | 81 | 99 | 97 | 3 |

**Estimates**

| Measure: | MEASURE_1 | |
|---|---|---|
| Evaluation_Moment | | |
| | Mean | Upper Bound |
| Sbp_T1 | 152,698 | 157,876 |
| Sbp_T2 | 152,921 | 158,258 |
| Sbp_T3 | 149,048 | 154,114 |
| Sbp_T4 | 153,794 | 159,638 |
| Sbp_T5 | 156,651 | 162,546 |

**Estimates**

| Measure: | MEASURE_1 | |
|---|---|---|
| Evaluation_Moment | | |
| | Mean | Upper Bound |
| Dbp_T1 | 85,222 | 88,305 |
| Dbp_T2 | 83,524 | 86,564 |
| Dbp_T3 | 77,937 | 80,547 |
| Dbp_T5 | 81,984 | 85,235 |
| Dbp_T5 | 82,921 | 85,721 |

**Estimates**

| Measure: | MEASURE_1 | |
|---|---|---|
| Evaluation_Moment | | |
| | Mean | Upper Bound |
| Beats/min_T1 | 67,302 | 70,810 |
| Beats/min_T2 | 67,302 | 70,041 |
| Beats/min_T3 | 67,714 | 71,081 |
| Beats/min_T4 | 73,794 | 78,244 |
| Beats/min_T5 | 70,460 | 73,500 |

**Estimates**

| Measure: | MEASURE_1 |
|---|---|
| Evaluation_Moment | |
| | Mean |
| Breaths/min_T1 | 14,300 |
| Breaths/min_T2 | 13,600 |
| Breaths/min_T3 | 13,600 |
| Breaths/min_T4 | 16,100 |
| Breaths/min_T5 | 13,600 |

**Estimates**

| Measure: | MEASURE_1 |
|---|---|
| Evaluation_Moment | |
| | Mean |
| SpO2%_T1 | 96,600 |
| SpO2%_T2 | 95,900 |
| SpO2%_T3 | 97,100 |
| SpO2%_T4 | 95,200 |
| SpO2%_T5 | 96,800 |

**Estimates**

| Measure: | MEASURE_1 | |
|---|---|---|
| Evaluation_Moment | | |
| | Mean | Upper Bound |
| Beats/min_T1 | 67,302 | 70,810 |
| Beats/min_T2 | 67,302 | 70,041 |
| Beats/min_T3 | 67,714 | 71,081 |
| Beats/min_T4 | 73,794 | 78,244 |
| Beats/min_T5 | 70,460 | 73,500 |

**Correlations**

| | | Sbp_mmHg_T2 | Dbp_mmHg_T2 | Beats_min_T2 | Breaths_min_T2 | Percent_SpO2_T2 |
|---|---|---|---|---|---|---|
| STAI_S_T1 | Correlation Coefficient | 0,126 | 0,197 | 0,064 | 0,069 | -0,134 |
| | Sig. (2-tailed) | 0,326 | 0,122 | 0,619 | 0,592 | 0,294 |
| | N | 63 | 63 | 63 | 63 | 63 |
| VAS_T2 | Correlation Coefficient | 0,005 | 0,049 | 0,007 | 0,143 | -0,054 |
| | Sig. (2-tailed) | 0,966 | 0,704 | 0,958 | 0,265 | 0,675 |
| | N | 63 | 63 | 63 | 63 | 63 |
| Cortsol2 | Correlation Coefficient | -0,093 | -0,186 | 0,073 | 0,013 | 0,040 |
| | Sig. (2-tailed) | 0,526 | 0,202 | 0,620 | 0,930 | 0,785 |
| | N | 49 | 49 | 49 | 49 | 49 |

APPENDIX 15: Image quality data to Cross-sectional study.

| ID | Movements >1 | Movements <0.5 | %Noise (SA) | Contrast (SA) | % Noise (VLA) | Contrast (VLA) | % Noise (HLA) | Contrast (HLA) |
|---|---|---|---|---|---|---|---|---|
| 03 | 0 | 10 | 31,7691 | 12,6970 | 31,5452 | 7,6093 | 31,2154 | 3,0398 |
| 04 | 0 | 10 | 31,6700 | 21,9820 | 30,6767 | 23,4997 | 29,8717 | 26,0480 |
| 05 | 0 | 12 | 31,1678 | 11,8283 | 29,4241 | 11,1768 | 30,6823 | 9,3080 |
| 06 | 0 | 10 | 33,1071 | 4,5482 | 31,1257 | 4,1201 | 31,6053 | 7,4232 |
| 07 | 0 | 6 | 32,5214 | 4,2176 | 30,9551 | 2,7989 | 30,7596 | 4,6260 |
| 08 | 0 | 0 | 32,1018 | 16,2323 | 30,2400 | 7,3342 | 29,6971 | 21,7296 |
| 09 | 0 | 12 | 32,6462 | 7,6543 | 30,4780 | 3,5774 | 30,1278 | 9,8970 |
| 10 | 0 | 16 | 32,1214 | 16,1532 | 30,1309 | 4,2015 | 28,9166 | 6,1458 |
| 11 | 2 | 12 | 30,3320 | 12,8973 | 29,5052 | 7,8864 | 29,4308 | 3,4091 |
| 01 | 2 | 16 | 34,1053 | 5,8386 | 32,4032 | 4,4882 | 32,1556 | 1,9022 |
| 12 | 0 | 8 | 28,4489 | 6,5734 | 27,2013 | 4,7387 | 26,8241 | 3,1898 |
| 13 | 0 | 14 | 32,9565 | 8,6174 | 31,3965 | 2,3687 | 31,3217 | 8,8300 |
| 14 | 0 | 16 | 32,1795 | 2,6221 | 30,1816 | 2,3613 | 29,1292 | 2,3595 |
| 15 | 0 | 22 | 33,5889 | 4,6096 | 32,3004 | 2,9302 | 32,4403 | 1,1496 |
| 17 | 0 | 15 | 32,5441 | 9,5079 | 29,6375 | 4,1524 | 29,4871 | 1,7830 |
| 18 | 0 | 24 | 29,9708 | 2,8937 | 27,9214 | 3,7211 | 28,2807 | 1,8708 |
| 19 | 0 | 2 | 31,3168 | 5,0235 | 29,6379 | 9,9506 | 29,5835 | 3,5277 |
| 20 | 2 | 13 | 29,7141 | 2,9280 | 29,0659 | 4,3245 | 29,5234 | 2,3853 |
| 21 | 0 | 18 | 29,5954 | 7,3454 | 27,6536 | 22,8057 | 27,3500 | 4,9431 |
| 22 | 0 | 13 | 33,5912 | 6,4957 | 31,4075 | 5,2469 | 31,6726 | 5,5994 |
| 23 | 0 | 16 | 32,7400 | 5,6412 | 30,8290 | 2,7791 | 30,3732 | 10,2461 |
| 26 | 2 | 16 | 32,7967 | 4,9287 | 30,7107 | 4,5550 | 31,0694 | 3,1540 |
| 27 | 0 | 0 | 30,2609 | 14,5540 | 29,5080 | 2,3571 | 28,9805 | 3,8597 |
| 28 | 0 | 8 | 31,3108 | 16,7942 | 30,9033 | 2,6570 | 29,1070 | 10,0171 |
| 29 | 0 | 0 | 32,3285 | 9,5650 | 30,7490 | 4,0964 | 31,1674 | 5,4337 |
| 30 | 0 | 4 | 33,0071 | 11,8183 | 31,9148 | 3,4946 | 32,2557 | 25,8341 |
| 31 | 0 | 8 | 31,7318 | 3,1269 | 30,6856 | 5,6859 | 30,5700 | 3,7112 |
| 16 | 0 | 14 | 31,0777 | 5,6941 | 30,5780 | 3,2848 | 31,1261 | 4,4677 |
| 24 | 0 | 2 | 31,5741 | 5,1494 | 31,3468 | 1,8381 | 29,6771 | 8,7623 |
| 25 | 0 | 16 | 31,2241 | 2,4754 | 29,4874 | 2,6545 | 29,4506 | 1,7302 |
| 34 | 0 | 6 | 28,1965 | 19,2289 | 28,2747 | 8,1764 | 28,2368 | 4,8808 |
| 35 | 0 | 14 | 32,5323 | 3,8618 | 30,6755 | 1,9286 | 31,6143 | 6,4480 |
| 36 | 0 | 8 | 31,8620 | 8,8731 | 31,1695 | 3,3772 | 30,9330 | 28,9882 |
| 37 | 2 | 10 | 32,8403 | 6,3927 | 31,8547 | 2,7281 | 31,4783 | 42,4165 |
| 38 | 0 | 4 | 35,2699 | 8,0200 | 33,1248 | 4,3512 | 31,7969 | 4,1631 |
| 40 | 8 | 18 | 33,4544 | 5,8348 | 31,0023 | 5,5338 | 30,8764 | 4,5052 |
| 32 | 0 | 2 | 33,9000 | 8,8554 | 31,8998 | 3,2236 | 31,2247 | 5,4006 |
| 33 | 0 | 6 | 31,6681 | 7,8383 | 30,3301 | 4,4464 | 29,9797 | 3,7186 |
| 39 | 2 | 16 | 34,8553 | 2,6027 | 33,8496 | 3,7400 | 33,4024 | 2,0445 |
| 45 | 0 | 18 | 30,6119 | 2,7390 | 30,1005 | 7,8944 | 30,6605 | 3,2736 |
| 46 | 0 | 4 | 29,9978 | 5,8066 | 28,6377 | 3,5963 | 28,6422 | 5,0234 |
| 42 | 0 | 9 | 31,9400 | 23,3824 | 29,7302 | 8,4055 | 29,8951 | 18,6447 |
| 43 | 2 | 11 | 32,2535 | 12,0994 | 31,2752 | 4,2140 | 31,8938 | 5,3214 |
| 41 | 0 | 8 | 31,8547 | 4,0493 | 29,6753 | 6,2589 | 30,0876 | 2,9167 |
| 44 | 0 | 6 | 32,3466 | 3,1306 | 29,7661 | 3,2235 | 29,8468 | 3,2779 |
| 49 | 6 | 13 | 31,3751 | 3,8921 | 30,1329 | 4,1618 | 29,9412 | 2,4192 |
| 50 | 0 | 11 | 33,5237 | 5,5056 | 32,1797 | 2,7167 | 31,6710 | 1,3613 |
| 51 | 0 | 12 | 30,7069 | 4,2195 | 28,3487 | 2,4823 | 28,0911 | 1,5477 |
| 52 | 0 | 12 | 28,1981 | 12,1632 | 27,9367 | 3,2560 | 26,8646 | 4,5014 |
| 53 | 0 | 19 | 31,8370 | 1,5823 | 30,4559 | 2,8110 | 30,9190 | 2,1390 |
| 54 | 2 | 18 | 30,9498 | 8,7560 | 30,4224 | 2,8107 | 29,8235 | 2,1385 |
| 48 | 0 | 14 | 31,4114 | 7,0943 | 29,8702 | 2,0363 | 30,1517 | 3,2774 |
| 55 | 0 | 12 | 31,7289 | 2,7651 | 30,5875 | 9,8883 | 30,6963 | 4,8448 |
| 56 | 2 | 12 | 32,0900 | 6,5586 | 30,9235 | 1,9626 | 29,7979 | 2,4675 |
| 57 | 0 | 14 | 31,9645 | 5,0692 | 30,3741 | 3,3817 | 31,2626 | 5,3432 |
| 58 | 0 | 12 | 32,2962 | 4,3070 | 32,3155 | 4,9739 | 30,7502 | 2,4800 |
| 59 | 4 | 12 | 31,5276 | 2,7714 | 31,1170 | 1,9393 | 31,5366 | 1,8584 |
| 64 | 14 | 20 | 32,1322 | 2,6611 | 30,5768 | 3,3009 | 30,0326 | 23,4606 |
| 60 | 0 | 12 | 31,7522 | 3,6470 | 29,9632 | 5,5135 | 30,2616 | 2,7385 |
| 61 | 4 | 6 | 28,4204 | 3,3775 | 28,2736 | 7,6038 | 27,5745 | 2,5554 |
| 62 | 0 | 8 | 32,7196 | 3,9051 | 31,6829 | 3,2439 | 31,4677 | 2,1571 |
| 65 | 0 | 9 | 32,5852 | 3,3723 | 32,4048 | 6,6074 | 31,1685 | 2,7188 |

APPENDIX 16: Image quality statistic results to Cross-sectional study.

| | | | LHA: %Noise | LHA: Contrast | LVA: %Noise | LVA: Contrast | SA: % Noise | SA: Contrast | STAI-S | VAS | Sbp (mmHg) | Dbp (mmHg) | Beats_ min | Breath s_min | SpO2% | Nº of movements |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spearman' s rho | LHA: %Noise | Correlation Coefficient | 1,000 | 0,100 | ,838** | 0,076 | ,769** | -0,126 | 0,205 | 0,084 | -0,009 | 0,181 | ,300* | -0,013 | -0,158 | 0,105 |
| | | Sig. (2-tailed) | | 0,439 | 0,000 | 0,556 | 0,000 | 0,330 | 0,110 | 0,515 | 0,945 | 0,158 | 0,119 | 0,923 | 0,220 | 0,416 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| | LHA: Contrast | Correlation Coefficient | 0,100 | 1,000 | 0,054 | 0,127 | -0,049 | ,568** | ,426** | 0,194 | 0,067 | 0,131 | 0,151 | 0,176 | -0,034 | -0,054 |
| | | Sig. (2-tailed) | 0,439 | | 0,675 | 0,326 | 0,705 | 0,000 | 0,001 | 0,132 | 0,603 | 0,311 | 0,240 | 0,171 | 0,793 | 0,679 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| | LVA: %Noise | Correlation Coefficient | ,838** | 0,054 | 1,000 | 0,148 | ,802** | -0,186 | 0,090 | -0,024 | -0,078 | 0,088 | ,314* | 0,060 | -0,020 | 0,046 |
| | | Sig. (2-tailed) | 0,000 | 0,675 | | 0,251 | 0,000 | 0,149 | 0,486 | 0,851 | 0,547 | 0,495 | 0,013 | 0,642 | 0,875 | 0,725 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| | LVA: Contrast | Correlation Coefficient | 0,076 | 0,127 | 0,148 | 1,000 | 0,072 | ,264* | 0,006 | -0,082 | 0,120 | 0,034 | 0,063 | 0,140 | -0,145 | 0,045 |
| | | Sig. (2-tailed) | 0,556 | 0,326 | 0,251 | | 0,578 | 0,038 | 0,961 | 0,528 | 0,353 | 0,793 | 0,624 | 0,277 | 0,262 | 0,731 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| | SA: %Noise | Correlation Coefficient | ,769** | -0,049 | ,802** | 0,072 | 1,000 | -0,248 | 0,139 | -0,087 | -0,132 | 0,093 | ,281* | -0,072 | -0,064 | -0,001 |
| | | Sig. (2-tailed) | 0,000 | 0,705 | 0,000 | 0,578 | | 0,052 | 0,280 | 0,502 | 0,305 | 0,473 | 0,027 | 0,580 | 0,623 | 0,992 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| | SA: Contrast | Correlation Coefficient | -0,126 | ,568** | -0,186 | ,264* | -0,248 | 1,000 | 0,108 | 0,134 | 0,082 | 0,107 | 0,174 | 0,150 | -0,051 | -0,073 |
| | | Sig. (2-tailed) | 0,330 | 0,000 | 0,149 | 0,038 | 0,052 | | 0,405 | 0,298 | 0,527 | 0,408 | 0,177 | 0,243 | 0,692 | 0,570 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| | STAI-S | Correlation Coefficient | 0,205 | ,426** | 0,090 | 0,006 | 0,139 | 0,108 | 1,000 | ,284* | 0,126 | 0,197 | 0,064 | 0,069 | -0,134 | -0,135 |
| | | Sig. (2-tailed) | 0,110 | 0,001 | 0,486 | 0,961 | 0,280 | 0,405 | | 0,024 | 0,326 | 0,122 | 0,619 | 0,592 | 0,294 | 0,290 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| | VAS | Correlation Coefficient | 0,084 | 0,194 | -0,024 | -0,082 | -0,087 | 0,134 | ,284* | 1,000 | 0,005 | 0,049 | 0,007 | 0,143 | -0,054 | 0,051 |
| | | Sig. (2-tailed) | 0,515 | 0,132 | 0,851 | 0,528 | 0,502 | 0,298 | 0,024 | | 0,966 | 0,704 | 0,958 | 0,265 | 0,675 | 0,690 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| | Sbp (mmHg) | Correlation Coefficient | -0,009 | 0,067 | -0,078 | 0,120 | -0,132 | 0,082 | 0,126 | 0,005 | 1,000 | ,416** | -,260* | -0,171 | -0,097 | 0,118 |
| | | Sig. (2-tailed) | 0,945 | 0,603 | 0,547 | 0,353 | 0,305 | 0,527 | 0,326 | 0,966 | | 0,001 | 0,040 | 0,180 | 0,450 | 0,356 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| | Dbp (mmHg) | Correlation Coefficient | 0,181 | 0,131 | 0,088 | 0,034 | 0,093 | 0,107 | 0,197 | 0,049 | ,416** | 1,000 | -0,010 | -0,159 | 0,009 | -0,020 |
| | | Sig. (2-tailed) | 0,158 | 0,311 | 0,495 | 0,793 | 0,473 | 0,408 | 0,122 | 0,704 | 0,001 | | 0,936 | 0,212 | 0,946 | 0,875 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| | Beats_min | Correlation Coefficient | ,300* | 0,151 | ,314* | 0,063 | ,281* | 0,174 | 0,064 | 0,007 | -,260* | -0,010 | 1,000 | 0,158 | 0,063 | -0,022 |
| | | Sig. (2-tailed) | 0,119 | 0,240 | 0,013 | 0,624 | 0,027 | 0,177 | 0,619 | 0,958 | 0,040 | 0,936 | | 0,215 | 0,622 | 0,864 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| | Breaths_min | Correlation Coefficient | -0,013 | 0,176 | 0,060 | 0,140 | -0,072 | 0,150 | 0,069 | 0,143 | -0,171 | -0,159 | 0,158 | 1,000 | -0,057 | 0,182 |
| | | Sig. (2-tailed) | 0,923 | 0,171 | 0,642 | 0,277 | 0,580 | 0,243 | 0,592 | 0,265 | 0,180 | 0,212 | 0,215 | | 0,654 | 0,153 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| | SpO2% | Correlation Coefficient | -0,158 | -0,034 | -0,020 | -0,145 | -0,064 | -0,051 | -0,134 | -0,054 | -0,097 | 0,009 | 0,063 | -0,057 | 1,000 | -0,057 |
| | | Sig. (2-tailed) | 0,220 | 0,793 | 0,875 | 0,262 | 0,623 | 0,692 | 0,294 | 0,675 | 0,450 | 0,946 | 0,622 | 0,654 | | 0,656 |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| | Nº of movements | Correlation Coefficient | 0,105 | -0,054 | 0,046 | 0,045 | -0,001 | -0,073 | -0,135 | 0,051 | 0,118 | -0,020 | -0,022 | 0,182 | -0,057 | 1,000 |
| | | Sig. (2-tailed) | 0,416 | 0,679 | 0,725 | 0,731 | 0,992 | 0,570 | 0,290 | 0,690 | 0,356 | 0,875 | 0,864 | 0,153 | 0,656 | |
| | | N | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 63 |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX 17: Socio-demographic data for Pilot Study.

| ID | Age | Gender | Prior Exams | Musical intervention choice | Music Volume | Acquisition time |
|---|---|---|---|---|---|---|
| 63 | 79 | F | S | 3 | 54 | 18 |
| 66 | 69 | F | S | 3 | 54 | 18 |
| 67 | 65 | F | N | 3 | 34 | 18 |
| 68 | 76 | F | N | 2 | 52 | 18 |
| 69 | 63 | M | N | 3 | 52 | 18 |
| 70 | 67 | F | N | 2 | 32 | 18 |
| 71 | 69 | M | S | 3 | 40 | 18 |
| 72 | 83 | F | S | 2 | 40 | 18 |
| 73 | 79 | F | N | 3 | 38 | 18 |
| 74 | 67 | F | N | 5 | 42 | 6 |
| 75 | 53 | F | N | 2 | 42 | 6 |
| 76 | 69 | F | S | 3 | 40 | 6 |
| 77 | 65 | M | S | 2 | 44 | 6 |
| 78 | 67 | M | S | 2 | 44 | 6 |
| 79 | 64 | F | N | 2 | 44 | 6 |
| 80 | 54 | M | N | 2 | 44 | 6 |
| 81 | 70 | M | S | 2 | 44 | 18 |
| 82 | 36 | M | N | 2 | 44 | 18 |
| 83 | 71 | M | N | 3 | 44 | 18 |
| 84 | 38 | M | S | 4 | 44 | 18 |
| 85 | 34 | M | S | 2 | 44 | 18 |
| 86 | 61 | M | S | 4 | 44 | 18 |
| 87 | 57 | M | S | 3 | 44 | 18 |
| 88 | 76 | M | S | 2 | 44 | 18 |
| 89 | 65 | F | S | 3 | 44 | 18 |
| 90 | 76 | M | N | 2 | 44 | 18 |
| 91 | 72 | M | S | 2 | 44 | 18 |
| 92 | 54 | F | N | 2 | 44 | 18 |
| 93 | 80 | F | N | 3 | 44 | 18 |
| 94 | 61 | M | S | 2 | 44 | 6 |
| 95 | 84 | F | N | 3 | 44 | 6 |
| 96 | 56 | F | N | 2 | 44 | 6 |
| 97 | 45 | M | S | 3 | 44 | 6 |

APPENDIX 18: STAI-S scores to Pilot study.

| ID | STAI-S T2 | | | | | | | | | | | | | | | | | | | | STAI-S T5 | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1- | 2- | 3- | 4- | 5- | 6- | 7- | 8- | 9- | 10- | 11- | 12- | 13- | 14- | 15- | 16- | 17- | 18- | 19- | 20- | 1- | 2- | 3- | 4- | 5- | 6- | 7- | 8- | 9- | 10- | 11- | 12- | 13- | 14- | 15- | 16- | 17- | 18- | 19- | 20- |
| 63 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 1 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 |
| 66 | 2 | 1 | 2 | 0 | 2 | 0 | 0 | 3 | 1 | 2 | 1 | 1 | 0 | 0 | 2 | 2 | 1 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 3 | 3 |
| 67 | 2 | 2 | 0 | 0 | 3 | 1 | 1 | 3 | 1 | 3 | 3 | 0 | 1 | 0 | 3 | 2 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 3 | 0 | 1 | 3 | 0 | 3 | 0 | 1 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 3 |
| 68 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 3 | 1 | 1 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 1 | 2 | 0 |
| 69 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 3 | 2 | 2 | 0 | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 |
| 70 | 3 | 3 | 0 | 0 | 3 | 0 | 2 | 2 | 1 | 3 | 3 | 1 | 1 | 0 | 3 | 2 | 2 | 0 | 2 | 3 | 3 | 3 | 1 | 1 | 3 | 0 | 2 | 3 | 0 | 2 | 3 | 1 | 1 | 0 | 3 | 3 | 2 | 0 | 2 | 3 |
| 71 | 0 | 2 | 3 | 0 | 3 | 2 | 1 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 3 | 3 | 1 | 2 | 0 | 3 | 0 | 0 | 2 | 3 | 3 | 0 | 0 | 1 | 0 | 3 | 2 | 2 |
| 72 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 73 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 1 | 2 | 2 | 3 | 2 | 1 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 0 | 1 | 2 | 0 | 1 | 3 | 0 | 2 | 2 | 1 | 1 | 0 | 2 | 2 | 1 | 0 | 2 | 2 |
| 74 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 75 | 3 | 2 | 2 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 3 | 1 | 0 | 2 | 0 | 0 | 3 | 0 | 3 | 2 | 1 | 0 | 0 | 2 | 2 | 1 | 0 | 2 | 3 |
| 76 | 3 | 3 | 3 | 0 | 3 | 0 | 1 | 2 | 0 | 2 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 |
| 77 | 2 | 2 | 1 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 1 | 2 | 3 | 1 | 1 | 2 | 0 | 0 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | 2 | 2 |
| 78 | 2 | 2 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 3 | 2 | 2 | 2 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| 79 | 3 | 3 | 0 | 1 | 1 | 0 | 1 | 2 | 0 | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 1 | 0 | 3 | 3 | 2 | 2 | 2 | 1 | 2 | 1 | 1 | 3 | 1 | 2 | 2 | 1 | 2 | 1 | 3 | 3 | 2 | 1 | 2 | 3 |
| 80 | 2 | 2 | 0 | 3 | 3 | 1 | 2 | 2 | 3 | 3 | 2 | 2 | 1 | 0 | 3 | 2 | 2 | 1 | 2 | 2 | 3 | 3 | 0 | 3 | 0 | 1 | 3 | 3 | 3 | 3 | 2 | 3 | 1 | 2 | 2 | 2 | 1 | 3 | 3 | 3 |
| 81 | 0 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 0 | 0 | 2 | 2 | 3 | 1 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 3 | 2 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 |
| 82 | 2 | 2 | 2 | 1 | 2 | 1 | 0 | 2 | 0 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | 2 | 2 | 2 | 2 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 3 | 3 | 1 | 1 | 1 | 2 | 2 | 2 | 0 | 2 | 2 |
| 83 | 2 | 3 | 1 | 0 | 2 | 1 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 1 | 2 | 2 | 0 | 0 | 2 | 2 | 3 | 3 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 3 | 0 | 0 | 0 | 3 | 3 | 2 | 0 | 2 | 3 |
| 84 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 0 | 0 | 3 | 0 | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 1 | 0 | 2 | 3 |
| 85 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 3 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 1 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 2 | 3 |
| 86 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 1 | 3 | 0 | 0 | 1 | 2 | 3 | 1 | 0 | 3 | 3 | 3 | 3 | 0 | 1 | 3 | 0 | 0 | 3 | 0 | 1 | 3 | 0 | 1 | 0 | 2 | 3 | 0 | 0 | 3 | 3 |
| 87 | 3 | 3 | 0 | 3 | 2 | 0 | 1 | 2 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 3 | 3 | 3 | 3 | 1 | 0 | 3 | 0 | 1 | 2 | 0 | 1 | 3 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 3 | 3 |
| 88 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 3 | 2 | 1 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 0 | 0 | 3 | 0 | 1 | 3 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 3 | 3 |
| 89 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 3 | 1 | 0 | 1 | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 2 | 1 | 1 | 1 | 2 |
| 90 | 3 | 3 | 0 | 1 | 2 | 0 | 1 | 3 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 3 | 1 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 3 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 0 | 0 | 2 | 2 | 0 | 0 | 1 | 2 |
| 91 | 2 | 2 | 1 | 2 | 2 | 0 | 0 | 3 | 2 | 2 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 |
| 92 | 3 | 3 | 2 | 1 | 2 | 2 | 0 | 3 | 0 | 2 | 3 | 1 | 1 | 0 | 2 | 2 | 0 | 2 | 0 | 2 | 3 | 3 | 0 | 1 | 3 | 0 | 1 | 2 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 3 |
| 93 | 2 | 3 | 3 | 2 | 3 | 1 | 3 | 2 | 1 | 2 | 2 | 3 | 2 | 0 | 1 | 3 | 2 | 0 | 2 | 2 | 3 | 2 | 2 | 0 | 3 | 0 | 2 | 3 | 0 | 2 | 2 | 2 | 1 | 0 | 1 | 3 | 2 | 0 | 2 | 3 |
| 94 | 2 | 1 | 3 | 2 | 2 | 1 | 3 | 2 | 2 | 2 | 2 | 3 | 2 | 1 | 2 | 2 | 3 | 2 | 1 | 2 | 2 | 2 | 1 | 0 | 3 | 0 | 1 | 3 | 0 | 3 | 2 | 0 | 0 | 0 | 2 | 3 | 1 | 0 | 2 | 2 |
| 95 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 1 | 3 | 3 | 1 | 1 | 0 | 3 | 3 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 1 | 0 | 0 | 3 | 0 | 3 | 2 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 3 |
| 96 | 2 | 3 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 3 | 3 | 1 | 0 | 3 | 0 | 0 | 3 | 0 | 2 | 2 | 0 | 0 | 0 | 3 | 3 | 2 | 0 | 3 | 3 |
| 97 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 3 | 3 | 1 | 1 | 3 | 0 | 1 | 3 | 0 | 3 | 2 | 0 | 0 | 1 | 2 | 3 | 0 | 0 | 3 | 3 |

APPENDIX 19: VAS scores for Pilot Study.

| ID | VAS_T2 | VAS_T3 | VAS_T4 | VAS_T5 |
|---|---|---|---|---|
| 63 | 2 | 0 | 0 | 0 |
| 66 | 6 | 3 | 0 | 0 |
| 67 | 2 | 0 | 7 | 5 |
| 68 | 2 | 2 | 1 | 1 |
| 69 | 7 | 3 | 3 | 5 |
| 70 | 5 | 5 | 0 | 0 |
| 71 | 8 | 4 | 7 | 5 |
| 72 | 2 | 1 | 1 | 1 |
| 73 | 10 | 3 | 5 | 1 |
| 74 | 5 | 1 | 2 | 4 |
| 75 | 5 | 3 | 3 | 1 |
| 76 | 0 | 0 | 0 | 0 |
| 77 | 8 | 4 | 2 | 0 |
| 78 | 3 | 2 | 0 | 0 |
| 79 | 5 | 3 | 0 | 0 |
| 80 | 6 | 3 | 2 | 2 |
| 81 | 7 | 3 | 8 | 6 |
| 82 | 7 | 3 | 1 | 0 |
| 83 | 0 | 0 | 0 | 0 |
| 84 | 7 | 2 | 1 | 1 |
| 85 | 5 | 0 | 0 | 3 |
| 86 | 4 | 2 | 0 | 0 |
| 87 | 5 | 0 | 5 | 1 |
| 88 | 8 | 1 | 1 | 0 |
| 89 | 3 | 0 | 3 | 5 |
| 90 | 3 | 0 | 2 | 0 |
| 91 | 6 | 1 | 3 | 0 |
| 92 | 0 | 0 | 0 | 0 |
| 93 | 8 | 6 | 0 | 2 |
| 94 | 5 | 2 | 7 | 1 |
| 95 | 0 | 0 | 0 | 0 |
| 96 | 7 | 2 | 4 | 3 |
| 97 | 2 | 0 | 3 | 1 |

APPENDIX 20: Physiological data for Pilot study.

| ID | Sbp(mmHg)_T2 | Dbp(mmHg)_T2 | Beats/min_T2 | Breaths/min_T2 | %SpO2_t2 | Sbp(mmHg)_T3 | Dbp(mmHg)_T3 | Beats/min_T3 | Breaths/min_T3 | %SpO2_T3 | Sbp(mmHg)_T4 | Dbp(mmHg)_T4 | Beats/min_T4 | Breaths/min_T4 | %SpO2_T4 | Sbp(mmHg)_T5 | Dbp(mmHg)_T5 | Beats/min_T5 | Breaths/min_T5 | %SpO2_T5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 63 | 167 | 81 | 53 | 14 | 98 | 174 | 79 | 60 | 11 | 97 | 185 | 88 | 54 | 17 | 95 | 173 | 82 | 56 | 13 | 98 |
| 66 | 146 | 10 | 85 | 15 | 92 | 159 | 97 | 81 | 10 | 98 | 163 | 97 | 76 | 17 | 98 | 167 | 107 | 79 | 12 | 98 |
| 67 | 129 | 85 | 75 | 12 | 94 | 146 | 88 | 70 | 10 | 85 | 140 | 82 | 71 | 13 | 96 | 164 | 96 | 80 | 14 | 96 |
| 68 | 155 | 103 | 95 | 14 | 93 | 131 | 108 | 93 | 12 | 93 | 95 | 57 | 99 | 16 | 94 | 140 | 81 | 92 | 13 | 92 |
| 69 | 131 | 81 | 85 | 15 | 90 | 118 | 81 | 87 | 12 | 95 | 107 | 73 | 90 | 14 | 93 | 124 | 81 | 87 | 12 | 96 |
| 70 | 126 | 75 | 75 | 14 | 96 | 134 | 76 | 84 | 11 | 96 | 165 | 106 | 88 | 15 | 92 | 138 | 75 | 79 | 13 | 92 |
| 71 | 143 | 114 | 90 | 14 | 93 | 149 | 100 | 88 | 11 | 94 | 161 | 104 | 114 | 16 | 92 | 144 | 102 | 90 | 12 | 95 |
| 72 | 183 | 101 | 72 | 13 | 92 | 196 | 91 | 71 | 11 | 94 | 198 | 114 | 94 | 15 | 98 | 197 | 110 | 77 | 13 | 96 |
| 73 | 147 | 77 | 65 | 14 | 92 | 179 | 77 | 63 | 11 | 93 | 174 | 76 | 71 | 16 | 96 | 165 | 74 | 66 | 13 | 97 |
| 74 | 117 | 75 | 56 | 12 | 96 | 141 | 81 | 52 | 10 | 96 | 123 | 70 | 63 | 14 | 90 | 139 | 79 | 58 | 12 | 96 |
| 75 | 117 | 68 | 81 | 13 | 99 | 111 | 80 | 90 | 11 | 78 | 103 | 50 | 95 | 15 | 96 | 104 | 74 | 84 | 11 | 98 |
| 76 | 164 | 79 | 83 | 14 | 95 | 178 | 93 | 75 | 12 | 94 | 153 | 93 | 86 | 15 | 93 | 142 | 83 | 74 | 11 | 95 |
| 77 | 139 | 58 | 82 | 13 | 98 | 177 | 41 | 69 | 11 | 98 | 90 | 42 | 81 | 14 | 91 | 82 | 70 | 80 | 10 | 97 |
| 78 | 190 | 101 | 89 | 12 | 95 | 190 | 103 | 83 | 10 | 98 | 177 | 91 | 83 | 16 | 89 | 164 | 91 | 82 | 11 | 98 |
| 79 | 133 | 87 | 91 | 13 | 98 | 142 | 78 | 79 | 11 | 97 | 101 | 65 | 88 | 15 | 88 | 128 | 68 | 87 | 12 | 92 |
| 80 | 185 | 93 | 58 | 14 | 98 | 147 | 82 | 64 | 12 | 98 | 149 | 95 | 66 | 17 | 88 | 147 | 81 | 63 | 11 | 94 |
| 81 | 187 | 117 | 68 | 13 | 97 | 169 | 94 | 71 | 11 | 98 | 160 | 92 | 60 | 16 | 90 | 152 | 92 | 73 | 12 | 96 |
| 82 | 115 | 88 | 77 | 15 | 97 | 124 | 88 | 87 | 11 | 93 | 123 | 92 | 74 | 15 | 89 | 120 | 75 | 70 | 14 | 92 |
| 83 | 120 | 79 | 86 | 17 | 96 | 131 | 85 | 76 | 10 | 95 | 145 | 90 | 70 | 16 | 91 | 139 | 83 | 67 | 12 | 94 |
| 84 | 123 | 69 | 38 | 18 | 97 | 117 | 70 | 39 | 13 | 97 | 125 | 80 | 51 | 16 | 91 | 125 | 75 | 43 | 11 | 97 |
| 85 | 107 | 71 | 72 | 14 | 93 | 105 | 76 | 68 | 12 | 97 | 115 | 74 | 72 | 15 | 89 | 112 | 76 | 73 | 12 | 94 |
| 86 | 149 | 79 | 71 | 14 | 96 | 146 | 74 | 79 | 12 | 92 | 133 | 71 | 80 | 18 | 91 | 143 | 80 | 76 | 13 | 97 |
| 87 | 153 | 90 | 67 | 12 | 95 | 150 | 91 | 63 | 11 | 97 | 160 | 99 | 74 | 19 | 92 | 162 | 91 | 69 | 12 | 95 |
| 88 | 133 | 76 | 80 | 14 | 97 | 150 | 82 | 81 | 11 | 93 | 152 | 79 | 81 | 17 | 93 | 156 | 83 | 87 | 12 | 98 |
| 89 | 130 | 76 | 70 | 15 | 96 | 130 | 76 | 70 | 12 | 97 | 144 | 85 | 75 | 19 | 90 | 145 | 86 | 74 | 11 | 97 |
| 90 | 135 | 90 | 83 | 15 | 98 | 139 | 88 | 85 | 12 | 98 | 149 | 92 | 92 | 15 | 80 | 151 | 89 | 51 | 12 | 90 |
| 91 | 147 | 64 | 83 | 14 | 96 | 143 | 81 | 84 | 12 | 77 | 177 | 100 | 86 | 17 | 99 | 192 | 98 | 63 | 12 | 94 |
| 92 | 127 | 72 | 74 | 12 | 98 | 132 | 72 | 69 | 11 | 98 | 134 | 86 | 83 | 16 | 87 | 139 | 101 | 79 | 11 | 99 |
| 93 | 147 | 78 | 100 | 13 | 99 | 149 | 71 | 94 | 11 | 96 | 151 | 80 | 104 | 15 | 74 | 147 | 73 | 87 | 12 | 95 |
| 94 | 108 | 54 | 80 | 14 | 97 | 98 | 42 | 75 | 12 | 90 | 106 | 43 | 79 | 16 | 87 | 110 | 52 | 73 | 12 | 97 |
| 95 | 149 | 85 | 88 | 13 | 96 | 135 | 79 | 71 | 11 | 95 | 140 | 83 | 80 | 15 | 88 | 166 | 94 | 74 | 11 | 90 |
| 96 | 140 | 102 | 75 | 12 | 97 | 141 | 91 | 77 | 12 | 97 | 126 | 85 | 79 | 16 | 79 | 132 | 81 | 76 | 11 | 94 |
| 97 | 195 | 105 | 81 | 18 | 97 | 176 | 98 | 81 | 12 | 88 | 180 | 110 | 96 | 16 | 80 | 175 | 90 | 80 | 12 | 92 |

APPENDIX 21: Statistic results to Pilot study.

| Acquisition time | | Mean | Std. Error | Interval Lower Bound | Interval Upper Bound |
|---|---|---|---|---|---|
| 6.00 | VAS_T2 | 4,182 | 0,821 | 2,508 | 5,855 |
| | VAS_T3 | 1,818 | 0,507 | 0,785 | 2,851 |
| | VAS_T4 | 2,000 | 0,744 | 0,485 | 3,515 |
| | VAS_T5 | 1,182 | 0,577 | 0,006 | 2,358 |
| 18.00 | VAS_T2 | 4,783 | 0,568 | 3,625 | 5,940 |
| | VAS_T3 | 1,696 | 0,351 | 0,981 | 2,410 |
| | VAS_T4 | 2,130 | 0,514 | 1,083 | 3,178 |
| | VAS_T5 | 1,522 | 0,399 | 0,708 | 2,335 |

**Estimates**

Measure:

| tempoExame | Mean | Std. Error | Interval Lower Bound | Interval Upper Bound |
|---|---|---|---|---|
| Sbp_T2 | 148,254 | 2,351 | 143,587 | 152,921 |
| Sbp_T3 | 147,377 | 2,280 | 142,849 | 151,904 |
| Sbp_T4 | 148,426 | 2,646 | 143,173 | 153,680 |
| Sbp_T5 | 150,870 | 2,518 | 145,870 | 155,869 |

Measure:

| Acquisition time | | Mean | Std. Error | Interval Lower Bound | Interval Upper Bound |
|---|---|---|---|---|---|
| 6.00 | STAI_T2 | 40,364 | 2,906 | 34,445 | 46,283 |
| | STAI_T5 | 31,182 | 3,044 | 24,981 | 37,383 |
| 18.00 | STAI_T2 | 39,478 | 2,010 | 35,385 | 43,572 |
| | STAI_T5 | 33,696 | 2,105 | 29,407 | 37,984 |

**Estimates**

Measure:

| tempoExame | Mean | Std. Error | 95% Confidence Interval Lower Bound | 95% Confidence Interval Upper Bound |
|---|---|---|---|---|
| Beats_T2 | 71,827 | 1,228 | 69,389 | 74,265 |
| Beats_T3 | 71,313 | 1,365 | 68,602 | 74,024 |
| Beats_T4 | 76,970 | 1,744 | 73,507 | 80,433 |
| Beats_T5 | 72,348 | 1,250 | 69,865 | 74,830 |

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Cortsol2 | 7,65645 | 22 | 6,442347 | 1,373513 |
| | Cortisol3 | 5,53655 | 22 | 3,600690 | 0,767670 |

**Estimates**

Measure:

| tempoExame | Mean | Std. Error | Interval Lower Bound | Interval Upper Bound |
|---|---|---|---|---|
| Dbp_T2 | 82,556 | 1,600 | 79,380 | 85,732 |
| Dbp_T3 | 80,292 | 1,259 | 77,792 | 82,791 |
| Dbp_T4 | 82,742 | 1,561 | 79,642 | 85,842 |
| Dbp_T5 | 83,666 | 1,221 | 81,243 | 86,089 |

**Estimates**

Measure:

| tempoExame | Mean | Std. Error | Interval Lower Bound | Interval Upper Bound |
|---|---|---|---|---|
| 1 | 95,863 | 0,490 | 94,891 | 96,835 |
| 2 | 95,563 | 0,390 | 94,789 | 96,338 |
| 3 | 92,829 | 0,600 | 91,638 | 94,021 |
| 4 | 96,053 | 0,329 | 95,400 | 96,706 |

**Estimates**

Measure:

| | | Mean | Std. Error | Interval Lower Bound | Interval Upper Bound |
|---|---|---|---|---|---|
| | Breaths_T2 | 13,941 | 0,267 | 13,410 | 14,472 |
| | Breaths_T3 | 11,294 | 0,253 | 10,792 | 11,796 |
| | Breaths_T4 | 15,824 | 0,922 | 13,993 | 17,654 |
| | Breaths_T5 | 12,029 | 0,267 | 11,499 | 12,560 |