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Ciclo XXXIV

INTERNAL AND DYSMETABOLIC COMORBIDITIES OF ANXIOUS-DEPRESSIVE SYMPTOMATOLOGY ACCORDING TO A PSYCHO-NEURO-IMMUNO-ENDOCRINOLOGICAL (PNEI) PERSPECTIVE

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“For this is the great error of our day that the physicians separate the soul from the body” (Hippocrates, sixth century B.C.)

ABSTRACT

Background and General Aim: Anxious and depressive symptoms are the most common emotional disorders in outpatients, frequently co-occuring with Metabolic Syndrome (MetS) and colorectal adenomas (CRAs). Studies on their comorbidity, mostly conducted on inpatients, reported conflicting findings. According to a psycho-neuro-immuno-endocrinological (PNEI) approach, chronic inflammation and dysregulation of the Kynurenine (Kyn) Pathway (KP) could represent the *trait d'union* between these conditions.

Description of the studies:

STUDY I – Aim: To measure prevalence and association of anxious-depressive symptoms with MetS and its components among primary care patients. **Methods:** Cross-sectional study. Anxiety and depression were assessed with the Hospital Anxiety and Depression Scale (HADS). **Results:** Among 210 primary care outpatients, 84 (40%) had anxiety, 30 (14.29%) had depression and 26 (12.38%) had anxious-depressive symptoms. 66 patients (31.43%) had MetS. Symptoms of anxiety were inversely associated to employment (OR=0.28, p=0.04) and positively to waist circumference (OR=2.07, p=0.03); both depressive (OR=0.17, p<0.01) and anxious-depressive symptoms (OR=0.20, p<0.01) were inversely associated to education level. **Conclusions**: A positive association was found between waist circumference, key component of MetS, and anxiety, suggesting the need to clinically manage them in a coordinated way.

STUDY II – **Aim:** To assess prevalence and associations between anxious-depressive symptoms, MetS, CRAs and inflammatory markers in outpatients according to a PNEI view. **Methods**: Cross-sectional study. CRAs, MetS, anxious-depressive symptoms, personality traits and inflammatory markers were measured in outpatients undergoing colonoscopy. **Results:** Among 62 patients (50% men), 45.2% had CRAs and 41.9% MetS. Anxiety and depressive symptoms were detected in 16 (32.7%) and 9 (18.4%) subjects. CRAs correlated to male sex (r=0.32; p=0.01), age (r=0.34, p<0.01), IL-6 (r=0.31; p=0.03) and MetS (r=0.28; p=0.03). MetS correlated to age (r=0.32; p<0.001) and IL-6 (r=0.37; p<0.01). **Conclusions:** Our data suggest a link among proinflammatory status, some psychological traits and metabolic parameters.

STUDY III – Aim: To explore gender differences in a sample of outpatients undergoing colonoscopy for screening procedures. **Methods:** MetS was assessed according to ATPIII and IDF criteria. For the psychometric assessment HADS and Temperament and Character Inventory (TCI) were used. **Results:** Among 126 outpatients (51.6% male), 51 (44%) had CRAs, 54 (47%) MetS, 41 (41.4%) anxiety, 22 (22.2%) depression and 13 (13.1%) anxious-depressive symptoms. Diastolic hypertension (OR=10.14, p<0.01) was directly associated with male sex. TCI Reward Dependence (OR=0.67, p=0.04) and HDL (OR=0.94, p=0.02)

were inversely associated with male sex. **Conclusions:** Several gender differences were detected, suggesting the need to implement different preventive and diagnostic strategies in both sexes.

STUDY IV – Aim: To explore the role of inflammatory cytokines and KP metabolites in the comorbidity between anxious-depressive symptoms and MetS. **Methods:** Serum concentration of hs-CRP, IL-6 and KP metabolites were measured using liquid chromatography. **Results:** Among 126 subjects, MetS (*vs.* non MetS) patients had different distribution of several KP metabolites. At the regression analysis, hs-CRP ($p<0.01$) and 3-hydroxy-KYN ($p<0.01$) were associated with depressive symptoms. **Conclusions**: Collected data suggest that inflammatory cytokines and KP metabolites are involved in MetS, anxiety and depression.

Overall Conclusions: Preliminary data were collected about a link between anxious-depressive symptoms and internal-dysmetabolic disorders, suggesting new perspectives for the prevention and treatment of these conditions.

Key words: anxiety; depression; metabolic syndrome; colorectal adenoma; PNEI.

ABSTRACT IN ITALIANO

Introduzione e scopi generali: La sintomatologia ansioso-depressiva è tra i disturbi emotivi più comuni, e spesso si manifesta in pazienti con Sindrome Metabolica (SM) e adenomi colorettali (CRAs). Studi sulla loro comorbilità, condotti per lo più in pazienti ospedalizzati, hanno riportato risultati contrastanti. In un'ottica psico-neuro-immuno-endocrinologica (PNEI), l'infiammazione cronica e alterazioni della via della Chinurenina (KP) potrebbero rappresentare il *trait d'union* tra queste condizioni.

Descrizione degli studi:

STUDIO I – Scopo: Misurare prevalenze e associazioni tra sintomi di ansia e/o depressione e SM e sue componenti in pazienti della Medicina Generale. **Metodi**: Studio trasversale. La presenza di ansia e depressione è stata misurata con HADS (Hospital Anxiety and Depression Scale). **Risultati**: Di 210 pazienti ambulatoriali (126 donne, 60%), 84 (40%) avevano sintomi di ansia, 30 (14.29%) depressione e 26 (12.38%) sintomi ansioso-depressivi. I sintomi di ansia si associavano negativamente allo stato lavorativo (OR=0.28, p=0.04) e positivamente alla circonferenza vita (OR=2.07, p=0.03). Sia i sintomi depressivi (OR=0.17, p<0.01) che ansioso-depressivi (OR=0.20, p<0.01) erano inversamente associati al grado di istruzione. **Conclusioni**: L'associazione positiva tra circonferenza vita e sintomi di ansia suggerisce l'utilità di gestire clinicamente queste due dimensioni in modo coordinato.

STUDIO II – Scopo: Descrivere secondo un'ottica PNEI le associazioni tra sintomi ansioso-depressivi, SM, CRAs e markers infiammatori in pazienti ambulatoriali. **Metodi**: Studio trasversale. SM, CRAs, sintomi ansioso-depressivi, tratti di personalità e markers infiammatori soni stati misurati in pazienti ambulatoriali sottoposti a colonscopia. **Risultati**: Di 62 pazienti (50% maschi), 45.2% avevano CRAs e 41.9% SM. Ansia e depressione sono stati rilevati in 16 (32.7%) e 9 (18.4%) soggetti. I CRAs correlavano con sesso maschile (r=0.32; p=0.01), età (r=0.34, p<0.01), IL-6 (r=0.31; p=0.03) e SM (r=0.28; p= 0,03). La SM correlava con età (r=0.32; p<0.01) e IL-6 (r=0.37; p<0.01). **Conclusioni**: I dati suggeriscono un legame tra stato infiammatorio e parametri metabolici.

STUDIO III – Scopo: Esplorare le differenze di genere in un campione di pazienti ambulatoriali sottoposti a colonscopia di screening. **Metodi:** La SM è stata valutata con i criteri ATPIII e IDF. Per la valutazione psicometrica sono stati usati HADS e TCI. **Risultati**: Tra 126 pazienti (51.6% maschi), 51 (44%) avevano CRAs, 54 (47%) SM, 41 (41.4%) ansia, 22 (22.2%) depressione e 13 (13.1%) sintomi ansioso-depressivi. L'ipertensione diastolica (OR=10.14, p<0.01) era associata al sesso maschile. TCI *Reward Dependence* (OR=0.67, p=0.04) e colesterolemia HDL (OR=0.94, p=0.02) erano inversamente associati al sesso maschile. **Conclusioni:** Sono state rilevate diverse differenze di genere, suggerendo la potenziale utilità di differenziare strategie preventive e diagnostiche per i due sessi.

STUDIO IV – Scopo: Misurare il ruolo di markers infiammatori e metaboliti della KP nella comorbilità tra sintomi ansioso-depressivi e SM. **Metodi:** La concentrazione sierica di hs-PCR, IL-6 e dei metaboliti della KP è stata misurata con cromatografia liquida. **Risultati**: I pazienti con SM (*vs.* senza SM) avevano una diversa distribuzione di vari metaboliti della KP. Alla regressione multipla, hs-PCR ($p<0.01$) e 3-idrossi-chinurenina ($p<0.01$) erano associati a sintomi depressivi. **Conclusioni:** I dati suggeriscono che le citochine infiammatorie e i metaboliti della KP sono coinvolti in SM, ansia e depressione.

Conclusioni generali: Sono stati raccolti dati preliminari su un legame tra sintomi ansioso-depressivi e disturbi internistici-dismetabolici, suggerendo nuove prospettive per la prevenzione e il trattamento di queste condizioni.

Parole chiave: ansia; depressione; sindrome metabolica; adenoma colorettale; PNEI.

List of Congress Presentations and Abstracts

1. **Rioli G**, Bonamici C, Mancini S, Mattei G, Alboni S, Sena P, Roncucci L, Fiore G, Pingani L, Ferrari S, Galeazzi GM. Differenze di genere nella comorbilità tra sintomatologia Ansioso-Depressiva, Sindrome Metabolica e Adenomi Colorettali. SOPSI (Società Italiana di Psicopatologia) XXVI National Congress, Rome, June 20th-23rd, 2022. J of Psychopathology, 2022, 28 (suppl.1), 68-69. Abstract n° P.02.29.
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4. **Rioli G**, Cherubini M, Balducci J, Ferrari S, Zerbinati L, Grassi L. Suicidal risk among patients assessed at the Modena Consultation-Liaison Psychiatry Service: prevalence data from a 6-month cross-sectional study. International Convention on Suicidology and Public Health, XVII edition, 2019, September 17-18th. Aula Magna Rettorato, La Sapienza University, Rome.
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1. Galeazzi GM, Mattei G, **Rioli G**. Editoriale. "Digital Mental Health". Rivista Sperimentale di Freniatria 2020, 2. Franco Angeli Editore.
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3. Bologna M, Pietralunga S, Santambrogio J, **Rioli G**. Editoriale. "Vittimizzazioni". Rivista Sperimentale di Freniatria 2020, 1. Franco Angeli Editore.

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LIST OF ABBREVIATIONS

3-HANA=3-hydroxyanthranilic acid

3HK=3-hydroxy-kynurenine

5-HT=5-hydroxytryptamine (serotonin)

ANA=anthranilic acid

ATPIII=Adult Treatment Panel III

ATPIII-MetS=diagnosis of MetS according to ATPIII criteria

AUT1=autophagy, ascending colon

AUT2= autophagy, descending colon

AUT3= autophagy, sigmoid-rectum

BMI= body mass index

CHOL=total cholesterol

CIRS=Cumulative Illness Rating Scale

CRAs=Colorectal Adenomas

CRC= Colorectal Cancer

CRP=C-Reactive Protein

DBP=diastolic blood pressure

FMD 1 min-3 min%= percentage flow mediated dilation index, 1st-3rd minute

FMD basal-1 min%=percentage flow mediated dilation index, 1st minute

FMD basal-3 min%= percentage flow mediated dilation index, 3rd minute

FPG=fasting plasma glucose

GP= General Practitioner

HADS=Hospital Anxiety and Depression Scale

HADS-A=Hospital Anxiety and Depression Scale, anxiety subscale

HADS-AD=Hospital Anxiety and Depression Scale, anxiety and depression total subscale

HADS-D=Hospital Anxiety and Depression Scale, depression subscale

HC=hip circumference

HDL=high-density lipoprotein cholesterol

HPA=hypothalamic-pituitary-adrenal

HR=heart rate

hs-CRP=high sensitivity C-Reactive Protein

IDF=International Diabetes Federation

IDF-MetS=diagnosis of MetS according to IDF criteria

IL-1=Interleukine-1

IL-6=Interleukine-6

IMT=intima-media thickness

KYN=kynurenine

KYNA=kynurenic acid

KP=kynurenine pathway

LDL=low-density lipoprotein cholesterol

LPS=Lipopolysaccharide

LS-qIMT=carotid intima-media thickness, left side

MetS=Metabolic Syndrome

MPO mean=MPO mean concentration (ascendent, descendent and sigmoid colon tracts)

Myel1 or MPO1=myeloperoxidase, ascending colon

Myel2 or MPO2=myeloperoxidase, descending colon

Myel3 or MPO3=myeloperoxidase, sigmoid-rectum

NCEP= National Cholesterol Education Program

OGTT= Oral Glucose Tolerance Test

PICA=picolinic acid

PNEI=psycho-neuro-immuno-endocrinology

QUIN=quinolinic acid

RS-qIMT=carotid intima-media thickness, right side

SBP=systolic blood pressure

SF36=Short Form-36 Items

SF36-CS=Short Form-36 Items Component Summary

SF36-MCS=Short Form-36 Items Mental Component Summary

SF36-PCS=Short Form-36 Items Physical Component Summary

TCI=Temperament and Character Inventory

TCI-C=Cooperativeness

TCI-HA=Harm Avoidance

TCI-NS=Novelty Seeking

TCI-RD=Reward Dependence

TCI-SD=Self-Directedness

TCI-ST=Self-Transcendence

TRG=triglycerides

TRP=Tryptophan

VLDL=very low-density lipoprotein cholesterol

WC=waist circumference

WHR=waist-to-hip ratio

XANA=xanthurenic acid

1. INTRODUCTION

1.1 The Psychosomatic Medicine's approach to medical-psychiatric comorbidity

Psychosomatic Medicine can be defined as a comprehensive and interdisciplinary framework for the assessment of psychological factors affecting individual vulnerability, as well as course and outcome of illness. It is a discipline aimed to realize a biopsychosocial consideration of patient care in clinical practice, integrating a psychological approach in the prevention, treatment and rehabilitation of medical diseases[1].

The German psychiatrist Johann Christian August Heinroth introduced the term "Psychosomatic" in 1818, but modern "psychosomatic medicine", neologism introduced by Felix Deutsch around 1922, developed in the first half of the past century. The concept of psychosomatic resulted from the confluence of two concepts having an ancient tradition in Western thought and medicine: those of psychogenesis of disease and holism[2]. The psychogenetic model characterised the first phase of development of psychosomatic medicine (1930–1960) and resulted in the concept of "psychosomatic disease", i.e. a physical illness believed to be caused by psychological factors. Then, George Engel, Zbigniew Lipowski and David Kissen laid the groundwork for the development, in the sixties, of the current psychosomatic view of the disease.

Engel developed a multifactorial model of illness[3], later called "bio-psycho-social" (Engel, 1977), considering illness as a result of interacting mechanisms at the biological, psychological and social-interpersonal levels. As a result, the study of every disease must include the individual, his body and his surrounding environment as essential components of the total system[4,5]. The clinical translation of this model is partly represented by the Consultation-Liaison Psychiatry (CLP) that, since the beginning of the last century, fits as an area of psychiatry within general hospitals, dealing with the psychiatric and psychosocial implications of medical diseases[6].

Lipowski gave a significant contribution in setting the scope, mission and methods of psychosomatic medicine[7], identifying its three interrelated features: a) it is a scientific discipline concerned with the study of the relationships of biological, psychological, and social determinants of health and disease; b) it embodies a holistic approach to the practice of medicine; c) it encompasses CLP[5]. Both Engel and Lipowsky criticised the obsolete notion of psychogenesis, since it was incompatible with the doctrine of multicausality, a key postulate of current psychosomatic medicine.

Kissen provided a better specification of the term "psychosomatic"[8], clarifying that the relative weight of psychosocial factors may vary considerably from one individual to another within

the same illness, underscoring the basic conceptual defect of considering diseases as homogeneous entities.

To date, psychosomatic medicine has developed rapidly over the last decades, combining its two traditions of integrated psychosomatics in internal medicine and focusing on psychotherapeutic and psychiatric methods in many clinical fields. Since March 2003[9], psychosomatic medicine has been officially recognised as a subspecialty of psychiatry by the American Board of Medical Specialties.

According to the psychosomatic view, psychosocial and biological factors mutually interact in the course and outcome of medical disease, determining the unique quality of the experience and attitude of every patient in any episode of illness. Some factors (such as healthy habits and psychological well-being) positively promote health, rather than merely reducing disease. Conversely, other factors, such as chronic stress[10,11], the presence of psychiatric as well as subclinical symptoms, low quality of life and unhealthy behaviour can affect individual vulnerability to all type of disease. Mental disorders, in particular, increase the risk for communicable and non-communicable diseases; at the same time, many health conditions increase the risk for mental disturbances, and comorbidity complicates recognition and treatment of medical disorders[10,12].

Depression has emerged as an extremely important source of comorbidity in medical disorders[13]. According to literary evidence, depression increases symptom burden and functional impairment and worsens prognosis for heart disease, stroke, diabetes mellitus, HIV, AIDS, cancer and other chronic illnesses[14,15,16]. Moreover, it is associated to increased health care utilisation[17], higher mortality (particularly in the elderly people) and higher disability[16]. Finally, depression has an adverse effect on biological and self-care (e.g., adherence to diet, smoking cessation, exercise, medications), increasing the risk for the development of medical comorbidities[13,18].

The relationship between anxiety disorders and comorbid medical illness, despite less investigated, has also been found to imply important clinical implications[19]. For example, anxiety is associated with high rates of medically unexplained symptoms and increased utilization of healthcare resources[15,20,21]. Moreover, anxiety disorders are strongly and independently associated with chronic medical illness[22,23], low levels of physical health-related quality of life and physical disability[23-26].

Interestingly, the general psychosomatic approach has resulted in a number of sub-disciplines within their own areas of application: health psychology, psycho oncology, psychonephrology, psycho-neuro-immuno-endocrinology (PNEI), psychoimmunology, psychodermatololgy and others. Such sub-disciplines have developed clinical services, scientific societies and medical journals.

1.2 The PNEI perspective

Psycho-neuro-immuno-endocrinology (PNEI) is a scientific field of study that investigates the link between the nervous system, the endocrine system, the immune system and psychological processes, and the correlations of their bidirectional communications and cross-talk with physical health[27].

The principles of PNEI were theorized and divulgated in the second half of the 20th century by Dr. Robert Ader and colleagues[28]. The PNEI innovative medical approach represents a paradigm shift from a strictly biomedical view of health and disease taken as hermetically sealed compartments to a more interdisciplinary one. The sentence *"For this is the great error of our day that the physicians separate the soul from the body"* (Hippocrates, sixth century B.C.) clearly represents the *primum movens* of Dr. Ader's studies against the traditional scotomized medical view which separated organs and systems as independent from the rest of the body[27]. Dr. Ader's research on the conditioning of the immune system by psychosocial factors become a cornerstone for studies that finally lead to a unified vision of the biological functions of the body[28,29]. After years of ostracism and diffidence, mind-body interactions are now well recognized by the scientific community, deeply studied in the medical literature and taught at most important medical schools[27].

According to the PNEI paradigm, neurological system (limbic system and namely hypothalamus in primis), endocrine system (e.g. hypophysis and receptor glands) and immunity system are part of an integrated self-regulation network that, through a bidirectional cross-talks, is aimed at maintaining a psycho-somatic homeostasis in response to endogenous and exogenous stimuli[30], as shown in Figure 1. This complex interplay is mediated by a wide network of cytokines, hormones, growth factors, neuropeptides collectively named signalling (or messenger) molecules which represents the fundamental language of this physiological cross-talk[27].

The state of health or disease of a whole body can be depicted by the fluctuations of signalling molecules circulating levels: if the fluctuations are outside the homeostatic range (upper or lower than the physiological limits) we consider this status as a pathologic one. Many factors, such as heredity, environment, personality traits, emotions and lifestyles, can influence these interactions[27].

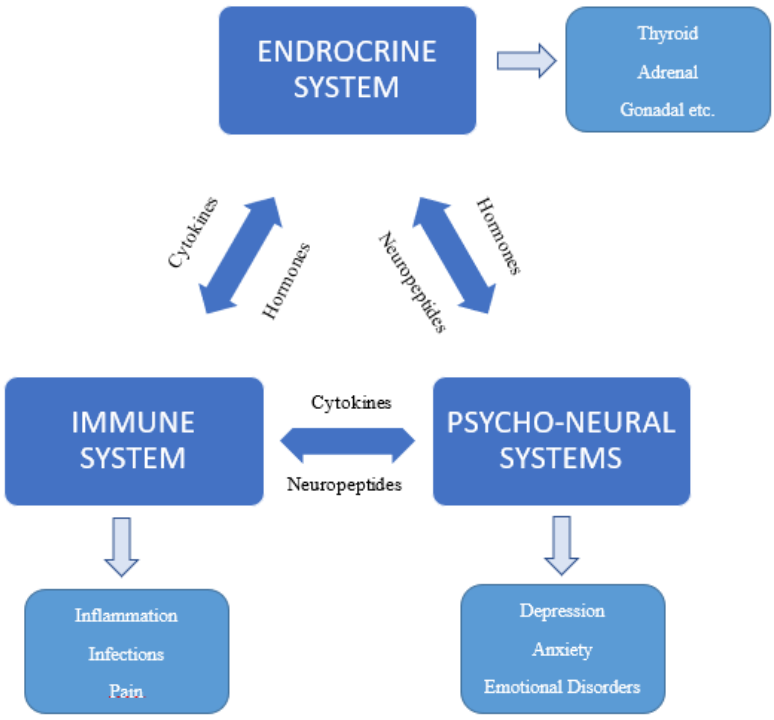


Figure 1. The interconnections among the PNEI systems.

Gut roles and relations with other organs and tissues are paradigmatic examples of the PNEI logic. Gut can be considered as complex immune and neuroendocrine organ integrated into the whole immune-endocrine systems and its correct functioning is crucial in order to guarantee the homeostasis and, consequently, the survival of the entire organism[31]. Insights into the gut-brain crosstalk have revealed a complex communication system that not only ensures the proper maintenance of gastrointestinal homeostasis, but is likely to have multiple effects on affect, motivation, and higher cognitive functions. The complexity of these interactions is enclosed in the denomination of "gut-brain axis" (GBA)[32].

The pivotal process through which the PNEI system intervenes in most diseases is undoubtedly chronic low grade inflammation[30,33]. Chronic stress, in fact, basically represents the persistent disequilibrium of the PNEI system pillars, and it acts on the hypothalamus-hypophysis axis and the whole endocrine system, which results finally in a modification of cortisol level. Stress consequently promotes tissue inflammation due to the increase of cortisol blood levels and the increase of inflammatory cytokines (i.e. IL-1, TNF, IL-6), activating the immune system in a pro-inflammatory sense[34]. PNEI describes how all these compartments influence and are influenced by the inflammatory processes to understand the close inter-relationship and pathogenesis of metabolic, immunologic, oncologic and psychiatric diseases[35, 304].

In the latest years, adopting an integrated PNEI perspective[304], an increasing number of studies is equating the weight of the risk factors related to psychosocial negative situations (depression

and anxiety in particular) to the classical biological ones (smoking, hypercholesterolemia, hypertension, obesity, diabetes) within the pathogenesis of cardiometabolic and internal diseases[36] and cancer[37].

1.3 Definition, epidemiology and pathogenesis of anxious-depressive symptoms

Definition

Depression and anxiety are the leading causes of disability worldwide[38], with high health care-related and social costs[39].

Depression refers to a wide range of mental health problems characterised by the absence of a positive affect (a loss of interest and enjoyment in ordinary things and experiences), low mood and a range of associated emotional, cognitive, physical, and behavioural symptoms. Distinguishing the mood changes between clinically significant degrees of depression (for example, major depression) and those occurring 'normally' remains problematic and it is best to consider the symptoms of depression as occurring on a continuum of severity[40]. The effects of depression can be long-lasting or recurrent and can dramatically affect a person's ability to function and live a rewarding life. The causes of depression are multifactorial, including complex interactions between social, psychological and biological factors; life events such as childhood adversity, loss and unemployment contribute to and may catalyse the development of depression[41,42].

Anxiety disorders are a cluster of mental disorders characterized by significant and uncontrollable feelings of anxiety and fear such that a person's social, occupational, and personal function are significantly impaired. There are several types of anxiety disorders, including generalized anxiety disorder, specific phobia, social anxiety disorder, separation anxiety disorder, agoraphobia, panic disorder, and selective mutism. The individual disorder can be diagnosed by the specific and unique symptoms, triggering events, and timing[43].

Epidemiology

Nowadays, depressive and anxiety disorders are becoming increasingly common in the general population[44], with higher prevalence detected among women. The lifetime prevalence of depression ranges from 20% to 25% in women and 7% to 12% in men[45]. The prevalence of anxiety among adult populations is the highest of any mental disorder, with more than one in four (28.8%) adults expected to meet diagnostic criteria during their lifetime[46]. Unfortunately, the vast majority of studies have focused on inpatient settings, therefore the real impact of mental disorders in outpatients are largely underestimated[47]. Comorbid depression and anxiety disorders occur in up to 25% of general practice patients[48], with high rates of comorbidity: about 85% of patients with depression have significant anxiety, and 90% of patients with anxiety disorder have depression[49]. Only less than half of these patients receive proper diagnosis and treatment[50].

Pathogenesis: role of inflammation

It is well known that alterations in neurotransmitter systems (mainly noradrenergic and serotonergic) and dysregulation of the HPA axis are involved in the pathophysiology of anxiety and depression[51].

In recent years, increasing evidence have suggested that the immune system in general, and chronic low grade inflammatory processes in particular, may contribute to anxious-depressive pathogenesis, both via direct actions on the brain as well as by effects on secondary pathways that marry brain to body[52-54].

The proinflammatory cytokines interleukin 6 (IL-6), tumor necrosis factor (TNF)-alfa and the acute phase reactant C Reactive Protein (CRP) are among the most reliable biomarkers of increased inflammation in anxious-depressed patients[55-57].

Evidence for this link is also found in studies of cancer patients and hepatitis C patients receiving immune-based therapy. These patients display increases in depressive tendencies while receiving interferon or interleukin-2 treatment, which subside once treatment finishes[58,59]. Furthermore, both clinical and preclinical studies have shown that the induction of a pro-inflammatory state in otherwise healthy subjects results in poor mood and "sickness behaviour", a behavioural phenotype resembling depression with symptoms including lethargy, anxiety, social withdrawal, anhedonia and anorexia[60,61].

Potential mechanisms for the inflammatory induction of behavioural changes may include effects of cytokines on neurotransmitter metabolism[62], HPA axis dysregulation[63], over-activation of microglia, impairments in synaptic plasticity[64] and alterations in blood brain barrier permeability[65].

Cytokines can also affect the kynurenine pathway (KP) in the brain by stimulating indoleamine 2,3-dioxygenase (IDO) production. As IDO is responsible for conversion of tryptophan (TRP) to kynurenine (KYN), the amount of TRP available for serotonin production is decreased and depressive-like behaviour is observed[66]. Pro-inflammatory cytokines may also increase kynurenine-3-mono-oxygenase enzyme activity, that degrades KYN into 3-hydroxykynurenine (3HK), shifting the KP from neuroprotection towards neurotoxicity with the production of neurotoxic metabolites and excitotoxicity[67, 300-303].

1.4 Medical comorbidities of anxious-depressive symptoms

Anxiety and depression are among the most common comorbidities of many chronic medical diseases including cancer and cardiovascular, metabolic, inflammatory and neurological diseases[68,69,19], accounting for a substantial part of the psychosocial burden of these disorders. Many factors can contribute to the occurrence medical-psychiatric comorbidities, such as shared genetic factors, converging biological pathways, social factors, health behaviours and psychological factors.

Diagnosis of anxiety and/or depression in patients with a medical disorder can be particularly challenging owing to symptomatic overlap. Although pharmacological and psychological treatments can be effective, adjustments may need to be made for patients with a comorbid medical disorder[68]. In addition, symptoms or treatments of medical disorders may interfere with the treatment of depression and/or anxiety. Conversely, symptoms of depression may decrease adherence to treatment of both disorders. Thus, screening for the presence of such conditions and comprehensive treatment plans are therefore crucial, especially in chronic outpatients, and liaison between different professionals according to a CLP perspective may represent a useful strategy to increase early detection and treatment[68].

In this thesis, we decided to focus on Metabolic Syndrome (MetS) and Colorectal Adenomas (CRAs) as examples of dysmetabolic and internal medical diseases highly prevalent in general population, and frequent comorbid with common emotional disorders such as anxiety and depression.

1.5 Definition, epidemiology and pathogenesis of MetS

Definition

MetS is a cluster of cardio-metabolic risk factors, affecting about a quarter of the world's adult population[70]. The specific components of MetS include central obesity, glucose intolerance, elevated triglycerides, low levels of high density lipoprotein cholesterol (HDL), and hypertension[71]. The components occur together more frequently than expected by chance, and when grouped together they result in an increased risk for cardiovascular disease and diabetes mellitus[72].

The prognostic implications of MetS are considerable: people with MetS have a doubled risk of dying of cardiovascular diseases and a tripled risk to suffer from heart attack or stroke, as well as all-cause mortality[72]. Moreover, most components of MetS have each been linked to the development of several types of cancer, including CRAs[73].

Since 1988, when Reaven first described it as "Syndrome X"[74], the definition and diagnostic criteria of MetS have been proposed and modified several times by different public health organizations. Both in research and clinical practice, two different sets of criteria are frequently referred to: the National Cholesterol Education Program, Adult Treatment Panel III criteria (NCEP, ATPIII)[75] and the International Diabetes Federation (IDF) criteria[70].

The ATPIII definition of MetS required three or more of the following five disorders: elevated waist circumference (≥102 cm in men and ≥88 cm in women), hypertriglyceridemia (≥1.7 mmol/l), low HDL cholesterol level (≤40 mg/dl in men and ≤50 mg/dl in women), high blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or pharmacological treatment), and elevated fasting glucose (≥100 mg/dl and/or

pharmacological treatment). The IDF definition used those same components and cut points, except for waist circumference cut points (≥94 cm in non-Hispanic white men and ≥80 cm in women), and requires obesity be prerequisite to diagnose MetS, thus emphasizing the role of visceral adiposity in the pathogenesis of the syndrome.

Epidemiology

The global prevalence of MetS differs depending on geographic and sociodemographic factors, as well as the diagnostic criteria used. The frequency of MetS is higher in Western countries and among people aged 40-80[76]. National Health and Nutrition Examination Survey data estimate that 35% of adults in the United States, and as much as 50% of the over-60 population, had a diagnosis of MetS (30.3% in men and 35.6% in women), based on the National Cholesterol Education Program Adult Treatment Panel III criteria[76]. European MetS prevalence, using International Diabetes Federation diagnostic criteria, has been estimated as 41% in men and 38% in women.

Pathogenesis: role of inflammation

The pathogenic mechanisms of MetS are complex and still not fully understood. Whether the individual components of MetS represent distinct pathologies or manifestations of a common pathogenic mechanism is still debated[77]. Visceral adiposity has been demonstrated to be a primary trigger for most of the pathways involved in MetS, thus stressing the importance of consumption of excess calories and lack of physical activity as major contributors. Of all the proposed mechanisms, insulin resistance, neurohormonal activation, and chronic systemic inflammation seem to play the crucial factors in the initiation, progression, and transition of MetS to CVD[77].

In comparison with acute inflammation, which is usually a physiological response to injury or infection, chronic inflammation in MetS, termed "metabolic inflammation", is characterized by low-level local or systemic inflammatory responses. As such, several studies have associated these conditions with increased circulating levels of acute phase proteins, TNF-α, C-reactive protein (CRP) and IL-6[78]. This sub-threshold inflammatory response might constitute the common pathophysiological background for all five MetS criteria[79,80].

Emerging evidence indicates that not only systemic inflammation, but also local gut inflammation plays a crucial role in the pathogenesis of MetS. Because of alterations in the intestinal microbiota composition, collectively referred to as "dysbiosis", and intestinal barrier breach, microbiota or its components (such as endotoxins) translocate into the circulation and further contribute to metabolic inflammation[81].

1.6 The controversial association between anxious-depressive symptoms and MetS

The clinical association between MetS, depression and anxiety has been critically discussed in the scientific literature, with contrasting findings[82].

Several studies revealed a positive association between MetS and depression[83], although individual risk varies dramatically across cohorts[84]. For example, one study found that participants with MetS had higher scores on the depression subscale of the Hospital Anxiety and Depression Scale (HADS), with waist circumference and HDL cholesterol showing significant and independent correlations[85]. Severity of depressive symptoms correlate with MetS or predict risk for its development among women[86]. According to the meta-analysis by Vancampfort and colleagues[87], individuals with major depressive disorders had a higher MetS prevalence and a higher risk for hyperglycemia and hypertriglicerydemia. Finally, the meta-analysis by Pan and colleagues[84] strongly supported a bidirectional association between MetS and depression. Conversely, other studies declared no association[88,89].

Epidemiological studies have repeatedly investigated the association between MetS and anxiety symptoms; however, the results have been inconsistent. A recent systematic review and meta-analysis of 18 observational studies[90] pointed out an association with MetS and anxiety, suggesting the importance of screening people affected by the latter for the presence of anxiety symptoms. On the contrary, other authors suggested a weak or failed to find an association among those with anxiety and a current MetS condition[83,88,90,91].

Finally, the association between MetS and concurrent symptoms of anxiety and depression is seldom addressed in literature, though being common in clinical practice[82,92].

Thus, this relationship remains incompletely understood, likely due to the heterogeneity of both MetS and anxiety and depression as complex, multifactorial disorders involving lifelong interplay between genes and environment[93,94].

As explained in the previous paragraphs, recent work has highlighted both MetS[79,80] and anxious-depressive conditions[95,97] as inflammatory conditions, involving both systemic and central immune cells and cytokines[96]. Therefore, it has been proposed that a low chronic systemic inflammation state might play a role in their comorbidity, with cytokines induced inflammation ultimately leading to dysregulation of the HPA axis, of the autonomic nervous system and of the inflammatory oxidative and nitrosative stress (IO&NS) pathways, together with alterations in the gut microbiota[97].

Particularly worth mentioning is that although these elements may have a huge impact, developmental and environmental factors are fundamental in determining the susceptibility to stress-related metabolic diseases. The way of perceiving the stressful event and the ability to contextualize it, along with previous life experiences, the possibility to exercise control over the stressor and the availability of social support determine a substantial difference in resiliency towards similar stressful conditions. Therefore, personality traits may play a pivotal role in mediating the association between MetS, anxiety and depression[98].

Finally, according to the PNEI perspective, not only systemic inflammation, but also intestinal inflammation and alterations in gut microbiota has been hypothesized to link MetS to anxious-

depressive symptoms. In fact, rather than being distinct systems, there is compelling evidence for bidirectional communication between gut and brain (the already mentioned "gut-brain axis", GBA), driven by neural, metabolic, endocrine and inflammatory mediators.

1.7 Definition epidemiology and pathogenesis of Colorectal Adenomas

Definition

Colorectal adenomas (CRAs) are proliferative dysplastic (premalignant) epithelial lesions that may show low- or high-grade dysplasia. Dysplasia is the term describing the histologic abnormality of an adenoma according to the degree of atypical cells. The dysplasia is categorized as low grade (mild), moderate, or high grade (severe). Cells showing high-grade dysplasia are similar to cells found in a carcinoma but are limited to the epithelium[99].

Epidemiology

The prevalence of CRAs increases with age and varies with geography and ethnicity. It has been estimated that >20% of Western populations have CRA. Patients with a history of CRAs are three to six times more likely to develop metachronous neoplasms (colorectal cancer, CRC, diagnosed at a later point in time) than are persons of the same age, gender, and race/ethnicity in the general population[100]. Therefore, early detection and removal of CRAs reduces risk of CRC, and early diagnosis and treatment of CRC likely increases survival[99].

Diagnosis

Several early detection tests for CRAs are available. The faecal occult blood test (FOBT) involves collection of three consecutive stools after consuming a specified diet and testing for the presence of blood, which may indicate either a colorectal adenoma or cancer. Sigmoidoscopy involves cleansing the descending and sigmoid colon and directly visualizing the lower half or third of the colorectum using a 60 cm endoscope. Colonoscopy involves cleansing the colorectum and directly visualizing the entire colon and rectum using a colonoscope[101].

Recent reports suggest that individuals with MetS have a higher risk of CRAs[102] and CRC[103-105]. Waist circumference and waist-to-hip ratio, which are indicators of abdominal obesity, a key component of MetS, are also strongly associated with CRC risk[106].

Pathogenesis: role of inflammation

CRC carcinogenesis is a complex, long-lasting multistep process, causally linked to various factors, including genetic predisposition, inflammatory bowel disease (IBD), lifestyle factors and nutrition. Both chronic intestinal inflammation, as observed in patients with IBD, and inflammatory processes in the gut microenvironment are known to promote CRAs development and CRC carcinogenesis[107,108].

There are three main ways in which inflammation can be associated with colorectal cancer (CRC): inflammation-associated tumorigenesis; tumour-elicited inflammation; therapy-

induced inflammation. In inflammation-associated tumorigenesis, chronic inflammation resulting from infections, dysregulated immune responses or environmental factors can initiate and promote tumorigenesis through the induction of DNA damage or epigenetic changes. In tumour-elicited inflammation, tumour progression initiates an inflammatory response that is often pro-tumorigenic owing to hypoxia-induced cell death or to breakdown of the epithelial barrier and the subsequent influx of microbial products. Similarly, therapy-induced inflammation can trigger tumour-promoting inflammation owing to the release of damage-associated molecular patterns (DAMPs) from necrotic cells. Furthermore, extrinsic factors, such as environmental exposure, dietary habits, and commensal and pathogenic microbiome composition, can directly and indirectly alter the behaviour of tumour cells, affecting disease progression and response to treatment[109].

1.8 The association between anxious-depressive symptoms and CRAs

Anxiety and depression are the most common psychological problems among CRC patients, with highly variable prevalence rates between studies. According to the review by Peng and colleagues, examining 15 studies on the prevalence of depression in CRC patients,11 of which also examined the prevalence of anxiety, the prevalence of depression ranging from 1.6% to 57% and the prevalence of anxiety ranging from 1.0% to 47.2%[110].

Depression and anxiety may be part of a reaction to the cancer diagnosis[111], but in many patients they will persist, causing an added burden during treatment and leading to more difficulty with general management and symptom control[112], increased duration of hospital stay[113], and decreased compliance with treatment[112]. Moreover, the presence of depression or anxiety in the patient is believed to influence survival rate[114] and to increase morbidity[115]. Therefore, having a good understanding on the prevalence and risk factors of anxiety and depression in these patients is mandatory to identify high-risk patients and to implement preventive strategies.

There is a longstanding notion that psychological factors may play a role in the aetiology of CRC[116,117]. However, empirical data are scarce, and the evidence of an association between depression, anxiety and cancer is inconclusive.

In a study among 81.612 women without prior cancer, the highest levels of depressive symptoms were associated to an elevated risk of incident CRC, especially in overweight women[118]. A prospective study examining whether anxiety and depression were associated with mortality risk among CRC patients, individuals with clinical depression had higher overall mortality risk after adjustment for relevant potential covariates[119].

Also the role of personality traits in CRC risk have been investigated[116] and, according to several studies, emotional suppression, inhibition and constraint could play a role in CRC carcinogenesis[120,121].

Several mechanisms have been hypothesized regarding the association between depression and CRC. Depression has been related to dysregulated immune function, including increased levels of the IL-6, TNF-alpha and CRP[55-57], all inflammatory markers which has been also linked to an increased CRC risk[123,124].

Alterations in cytokine secretion and regulation are another possible pathway through which exposure to depression may be related to CRC risk[125]. Another plausible pathway involves the HPA axis, because hormones produced during the activation of the HPA axis in response to chronic stress can promote tumorigenesis[126].

Depression has also been linked to unhealthy behaviour, such as smoking, physical inactivity, and excessive energy intake, all well-known risk factors for colorectal carcinogenesis[127]. Finally, depressive symptoms may influence the risk of CRC through adverse effects on metabolic control, through hyperinsulinemia, central adiposity and cortisol overproduction[118].

To the best of our knowledge, the specific impact of anxiety and depression on CRAs has yet to be determined. According to our preliminary previous research, personality traits may be involved in colorectal carcinogenesis not only influencing lifestyle features involved in carcinogenesis, but also attitudes toward screening procedures which may make detection of precancerous lesions more likely[128]. Literary results from publications on inflammatory markers of CRP, IL-6 and TNF-α as possible mediators of these associations are not consistent[122,123], and need to be further investigated.

Given the role of inflammation in CRAs and anxious-depressive psychopatology[122], it is reasonable to think that chronic systemic inflammation could eventually mediate the association between anxiety, depression and CRAs.

In summary, this introductory section showed the burden of anxious-depressive symptoms, MetS and CRAs in terms of epidemiological impact, morbidity and disability, on the general population, and, at the same time, investigated the main pathophysiological features of these conditions. Even more, we addressed the complexity of the interrelations between anxious-depressive symptoms, MetS and CRAs, showing that, according to a multidisciplinary PNEI perspective, a chronic systemic low grade inflammation state could represent the *trait d'union* between all these conditions.

2. AIMS OF THE THESIS

Given the global burden of anxiety and depression, MetS and CRAs on the general population, among the most common and debilitating disorders worldwide, along with their high rates of comorbidity, it is mandatory an effort aimed at assessing their association in outpatients and furnishing new insights into the mechanisms involved in their co-occurrence.

Studies support the hypothesis of an association between MetS, anxiety and/or depression, but results and inconsistent, and mostly based on clinical in-patient samples. Otherwise, studies on the association between anxious-depressive symptomatology and CRAs are lacking.

Recent work has highlighted anxious-depressive symptoms, MetS and CRAs as inflammatory conditions, involving both systemic and central immune cells. However, the mechanisms of their association are far to be understood.

Several studies support the hypothesis that a chronic low grade systemic inflammation state could represent the *trait d'union* between anxiety and depression and MetS. It seems reasonable to hypothesize that inflammation could play a role also in the comorbidity between anxious-depressive symptoms and CRAs. Moreover, according to a psychosomatic and PNEI perspective, both intestinal dysbiosis and local inflammatory changes in the colonic mucosa and personality traits could play a role in these comorbidities. However, few data have been produced so far about their relative impact on the whole body and on body-mind interplay.

Thus, overall aims of this thesis are:

1) to measure prevalence, correlations and associations of anxiety and depressive symptoms with MetS and its single components among primary care patients attending their General Practitioner (GP) clinics;
2) to describe the prevalence and association of anxious-depressive symptoms, MetS and CRAs in outpatients undergoing colonoscopy according to a PNEI perspective;
3) to investigate gender differences in anxious and/or depressive symptoms and personality traits among a sample of outpatients undergoing colonoscopy;
4) to assess the role of circulating pro-inflammatory cytokines and metabolites of the Kynurenine pathway (KP) in anxious and/or depressive symptoms and MetS among outpatients undergoing colonoscopy.

3. STUDIES

3.1 STUDY I

Association between anxious-depressive symptoms and Metabolic Syndrome and its single components: an Italian cross-sectional study among primary care outpatients.

These original data have been accepted for publication as Original Article:
Rioli Giulia, Mattei Giorgio, Bursi Serena, Padula Maria Stella, Pingani Luca, Ferrari Silvia, Galeazzi Gian Maria. Association between anxious-depressive symptoms and Metabolic Syndrome and its single components: an Italian cross-sectional study among primary care outpatients. Accepted for publication as Original Article in Minerva Psychiatry on January, 12th, 2022. Manuscript number: Minerva Psychiatry-2315.

ABSTRACT

Background: The association between anxious-depressive disorders and Metabolic Syndrome (MetS) has been seldom addressed in literature, despite being common in clinical practice, and with mixed results. Aim of this study was to measure prevalence and association of symptoms of anxiety and/or depression with MetS and its single components among primary care patients.

Methods: Cross-sectional study. The Hospital Anxiety and Depression Scale (HADS) was used to measure symptoms of anxiety and/or depression.

Results: Among 210 primary care outpatients (60% women), 84 (40%) had symptoms of anxiety, 30 (14.29%) had depressive symptoms and 26 (12.38%) had anxious-depressive symptoms; 66 patients (31.43% of the total sample) had MetS according to the Adult Treatment Panel III (ATPIII) definition, and 69 (37.62%) according to International Diabetes Federation (IDF) criteria. Anxious ($p<0.01$), depressive ($p<0.01$) and comorbid anxious-depressive ($p<0.01$) symptoms were significantly prevalent in female *vs.* male patients. At the multivariate logistic regression analysis, symptoms of anxiety were inversely associated to employment status (OR=0.28, p=0.04), and positively associated to waist circumference (ATPIII criteria) (OR=2.07, p=0.03); both depressive (OR=0.17, $p<0.01$) and anxious-depressive symptoms (OR=0.20, $p<0.01$) were inversely associated to education level.

Conclusion: At the regression analysis, a positive association was found between waist circumference, a key component of MetS, and anxiety symptoms, suggesting the need to clinically manage these two dimensions in a coordinated way. Further research is needed to better understand the causative pathways of this correlation.

3.1.1 INTRODUCTION

Symptoms of anxiety and depression are highly prevalent in primary care settings. Up to 24-50% of people attending their General Practitioner (GP) present a psychiatric disorder, the most common being Major Depressive Disorder (10.40%) and Generalized Anxiety Disorder (7.90%)[44]. Noticeably, less than 50% of those disorders are recognized and properly treated[50]. Particularly, severe anxiety and depressive symptoms could significantly impact on primary care patients' quality of life[129] and on medication adherence and treatment outcomes of comorbid medical conditions[130]. Moreover, they may lead to excessive and inappropriate use of health care resources[17].

Metabolic Syndrome (MetS) is a cluster of heart disease risk factors including high blood pressure, obesity, high blood sugar and dyslipidaemia. It affects more than 25% of the adult population worldwide, increasing the risk of stroke, heart attack, cardiovascular and all-cause mortality[70]. Currently, among the several clinical definitions of MetS, the Adult Treatment Panel III (ATPIII) and the International Diabetes Federation (IDF) are the most used criteria. According to ATPIII criteria, the diagnosis of MetS requires the presence of at least three of the following five conditions: waist circumference ≥ 88 cm in women or ≥ 102 cm in men; diastolic blood pressure (DBP) ≥ 85 mmHg or systolic blood pressure (SBP) ≥ 130 mmHg or on-going antihypertensive drug treatment; fasting glucose ≥ 100 mg/dl or insulin resistance or on-going antidiabetic drug treatment; fasting triglyceride (TRG) level ≥ 150 mg/dl or on-going treatment for this lipid abnormality; fasting high-density lipoprotein (HDL) cholesterol level < 50 mg/dl in women or < 40 mg/dl in men or drug treatment for low HDL[71]. According to the IDF definition, the diagnostic criteria for MetS must include central obesity (defined as waist circumference ≥ 80 cm in women or ≥ 94 cm in men in Europid subjects, with other specific levels for other ethnic groups) along with any two of the following conditions: TRG > 150 mg/dL or specific anti-TRG drug treatment; HDL cholesterol < 50 mg/dL in females or < 40 mg/dL in males , or specific HDL treatment; SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or hypertension treatment or previously diagnosed hypertension; raised fasting plasma glucose ≥ 100 mg/dL or previously diagnosed type 2 diabetes or treatment for elevate glucose[70].

The association between anxious-depressive disorders and MetS has been seldom addressed in literature, despite being common in clinical practice, and with mixed results. While some authors found an association between depression and MetS[83,84,131], others did not find any significant association[88,89]. As far as anxiety is concerned, some papers detected an association between anxiety and MetS[132,133], while others did not replicate this finding[134,90]. According to our previous study on a smaller sample (129 patients), co-occurrent anxious-depressive symptoms, but not anxiety and depression alone, were significantly associated with ATPIII-defined MetS[135].

Recent evidence suggests that a chronic low-grade systemic inflammatory state might represent the pathophysiologic link in the comorbility between symptoms of anxiety and depression and MetS. According to this hypothesis, pro-inflammatory cytokines could lead to the chronic activation of the autonomic nervous system and of the Hypothalamus-Pituitary-Adrenal (HPA) axis, causing an increased Inflammatory Oxidative and NitroSative (IO&NS) damage of both neural and peripheral cells[136,137]. From "comorbid disorders" to "path-shared disorders", MetS and its single components and anxious-depressive conditions may reciprocally influence each other[82].

Therefore, this study aimed to assess prevalence and measures of association between symptoms of anxiety and depression and MetS and its single components in a sample of primary care outpatients attending their GP practice.

3.1.2 MATERIAL AND METHODS

Ethics

The present study was approved by the Local Ethic Committee of the Province of Modena, Italy, and was conducted according to the Declaration of Helsinki for ethical standards in medical research and to the Good Clinical Practice. Only patients providing written informed consent were enrolled in the study.

Study Design

This study was conducted adopting a cross-sectional design. Preliminary results referring to a smaller sample (129 patients) of the same cohort of patients have been previously published[135]. In the present paper, we report the results of the complete data collection and analysis from the final sample size of 210 patients.

Study population

Outpatients attending 5 GP clinics in Modena, Italy, in a two months-period in 2013, were asked to participate in the study. To contact GPs, a snowball sampling was adopted, according to the methodology already described in our previous paper[135]. The patients' enrolment in the study and the clinical assessment of each patient were individually performed by three researchers (GR, GM and SB) who attended at the GP clinics during opening times.

Inclusion criteria

Male and female patients, aged between 40 and 80 years, were included in the study.

Exclusion criteria

The following exclusion criteria were adopted: patients affected by conditions (disorders or related treatments) involving the immune response (e.g. corticosteroids); patients being prescribed with antidepressants or second-generation antipsychotics; patients affected by major depressive disorder, addictions or psychosis (schizoaffective, bipolar, schizophrenic, organic or other psychotic disorder, according to DSM-5 or ICD-10 criteria); patients with

previous stroke, heart attack or other cardiovascular diseases; patients affected by obesity caused by hereditary conditions (e.g. Praeder-Willy syndrome) or type 1 diabetes; ongoing pregnancy.

Collected variables

The following clinical and non-clinical variables were collected, either directly on patients or by consulting their medical record:

1) socio-demographic (age, sex, marital status) and life-style related variables (occupation, level and years of school education, alcohol and smoking habits);

2) anxiety and depressive symptoms;

3) MetS-related variables, specifically: BMI (kg/m^2), waist circumference (cm), waist-to-hip ratio, SBP and DBP (mmHg), collected from each patient by researcher's direct assessment; fasting plasma glucose (mg/dl), HDL cholesterol (mg/dl) and TRG (mg/dl) collected from routine laboratory testing of the last six months;

4) ongoing medications, index of physical comorbidity.

The index of physical comorbidity was calculated according to the MetS-related medication profile of patients, namely ongoing prescription of statins and/or anti-hypertensive and/or anti-diabetic medications, in accordance with the diagnostic criteria of MetS[70,71]: patients with no such prescription, or prescription of one or two of these medications were considered to have a low index of comorbidity, patients with prescription of three or more were considered to have a high index.

Psychometric assessment

The Italian validated version of the Hospital Anxiety and Depression Scale (HADS) was used to assess the presence and the severity of symptoms of anxiety and/or depression[138,139]. This 14-items valid and reliable instrument is made of 7 questions for anxiety and 7 questions for depression, finally providing one score for anxiety (HADS-A) and one for depression (HADS-D). In order to perform the logistic regression analysis, the dependent variable was operationalized as follows: 0=no symptoms (HADS-A or HADS-D < 8), 1=symptoms present (HADS-A or HADS-D ≥ 8).

Metabolic and anthropometric variables

Body mass index (BMI, kg/ m^2) as a measure of body fat, based on height and waist, was manually calculated. Waist circumference was measured on the midpoint between the iliac crest and the lower costal margin of the last rib. Hip circumference was assessed by a tape measure at the level of the largest protrusion of the buttocks. Waist-to-hip ratio (WHR) was calculated as the ratio of the waist circumference to the hip circumference. All these anthropometric parameters were calculated to improve discrimination between Visceral Adipose Tissue (VAT) and other types of fat tissue, which may be only approximate if the BMI alone is used[140]. Arterial SBP and DBP (mmHg) were measured by means of a

sphygmomanometer. Records of the at least 6 months laboratory tests including TRG (mg/dl), HDL cholesterol (mg/dl) and glucose blood levels (mg/dl), provided data to assess the presence of MetS, both according to ATPIII and IDF definitions.

Confounders

Possible confounders were collected, namely: age (dichotomized according to the median age in the total sample), smoking (0=no; 1=yes), alcohol intake (dichotomized as: 0=0-2 alcoholic units per day; 1=3 or more alcoholic units per day), and chronic somatic disease. The latter was operationalized by means of an ad hoc index of comorbidity, defined as follow. For each patient, current use of statins, antihypertensive and anti-diabetes medications was recorded. Patients who were not taking such medications, or who were taking one irrespectively from the class, were considered to have a "low" index of comorbidity, while patients who were taking two or more were considered at "high" index of comorbidity. Considered the relevance of the socio-economic determinants of health and disease, also marital status (dichotomized as: 0=single or divorced or widow; 1=married or in a stable relationship), work condition (0=unemployed or housewife; 1= employed or retired), school education (dichotomized according to the median of the years of school attended) were collected as possible confounders.

Statistical analysis

All data, appropriately anonymized, were collected in a Microsoft Excel 2010 sheet developed for the purpose.

STATA 14.2 (College Station, Texas) was used to perform the statistical analysis. Continuous data were described using mean, standard deviation, median and range (minimum and maximum). Dichotomous data were summarized by means of frequencies and percentages. Student's t-test was used for means' comparison between men and women. The correlation analysis was performed by means of Pearson's χ^2. Three multivariate stepwise logistic regression analyses were performed using the HADS-A (presence/absence of anxiety symptoms), HADS-D (presence/absence of depressive symptoms) and HADS-AD (presence/absence of co-occurring anxious-depressive symptoms) scores as dependent variable, respectively. The usual level of statistical significance ($p < 0.05$) was set.

3.1.3 RESULTS

The sample was made up of 210 patients, 84 men (40% of the total sample, with a mean age of 60.7 ± 10.70 years, range 40-79 years) and 126 women (60% of the total sample, with a mean age of 61 ± 11.03 years, range 40-80 years).

Table 1 shows the main features of the sample as far as dichotomous variables are concerned, and Table 2 displays the characteristics of the continuous variables both in the total sample and among men and women.

Table 1. General features of the sample (dichotomous variables).

Covariables		***N***	***%***
Sex	Male	84	40
	Female	126	60
Age (years)	<62	109	51.90
	<62	101	48.10
Smoking Habit	No/ex-smoker	169	82.44
	Yes	36	17.56
Alcohol Use	No	178	87.25
	Yes	26	12.75
Work condition	Unemployed or housewife	19	9.05
	Employed/retired	191	90.95
Scholarity (range: 5-18 years)	<10 years	105	50
	>=10 years	105	50
Family status	Married/stable relationship	158	75.24
	Single	52	24.76
Anxiety	No	126	60
	Yes	84	40
Depression	No	180	85.71
	Yes	30	14.29
Anxiety and depression	No	184	87.62
	Yes	26	12.86
BMI (Kg/m^2)	<26.47	95	45.24
	>26.47	115	54.76
BMI - overweight	Yes	80	38.10
	No	130	61.90
BMI - obesity	Yes	62	29.52
	No	148	70.48
WHR	Gynoid	13	6.19
	Android	197	93.81
HR (>69 bpm)	Yes	108	51.43
	No	102	48.57
Index of comorbidity	Low	163	77.99
	High	46	22.01

Anti-cholesterol on-going therapy	Yes	49	23.44
	No	160	76.56
Anti-hypertensive on-going therapy	Yes	98	46.89
	No	111	53.11
Antidiabetic on-going therapy	Yes	20	9.57
	No	189	90.43
MetS criteria (ATPIII)	Yes	66	31.43
	No	144	68.57
MetS criteria (IDF)	Yes	79	37.62
	No	131	62.38
MetS individual components fulfilled (dychotomic)		***N***	***%***
HDL cholesterol <40 mg/dl in Men or <50 mg/dl in Women or on-going drug treatment	Fulfilled	53	25.36
	Unfulfilled	156	74.64
Glycemia ≥100 mg/dl or T2DM	Fulfilled	48	22.86
	Unfulfilled	162	77.14
Hypertriglyceridemia ≥150 mg/dl or on-going drug treatment	Fulfilled	62	29.52
	Unfulfilled	148	70.48
Hypertension (SBP ≥ 130 mmHg and/or DBP ≥ 86 mmHg or on-going drug treatment)	Fulfilled	122	58.37
	Unfulfilled	87	41.63
Waist circumference (ATP-III: ≥88 cm in Women, ≥102 cm in Men)	Unsatisfied	85	40.67
	Satisfied	124	59.33
Waist circumference (IDF: ≥80 cm in Women, ≥94 cm in Men)	Unsatisfied	45	21.53
	Satisfied	164	78.47

Abbreviations' list: BMI, Body Mass Index; WHR, Waist-to-Hip Ratio; HR, Heart Rate; bpm, beats per minute; MetS, Metabolic Syndrome; ATPIII, Adult Treatment Panel III; IDF, International Diabetes Federation; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High Density Lipoprotein Cholesterol; T2DM, type 2 Diabetes Mellitus.

As shown in Table 2 and in Figure 1, at the Student's t-test, the mean values of waist circumference (t=2.64, p<0.01) and waist to hip ratio (t=3.25, p<0.01) were statistically significant higher in men than in women; HADS-A score (t=-2.94, p<0.01), HADS-D score (t=-2.53, p<0.01) and HADS-AD score (t=-3.02, p<0.01) were significantly higher in women than in men, so the mean differences were not likely due to chance.

Table 2. Continuous variables in the total sample and in comparisons (t-test) between males and females, expressed in mean1±SD1/mean2±SD2 (Student's t-test, *p* value).

Continuous Variable (range)	**Total sample (mean±SD)**	**Gender**			
		Male (mean ±SD)	**Female (mean ±SD)**	**t**	**p**
Age, years (40-80)	60.88±10.88	60.70±10.70	61.01±11.03	-0.20	0.84
BMI, Kg/m^2 (17.02-47.26)	27.37±4.71	27.45±3.83	27.32±5.23	0.20	0.84
Waist, cm (61-192)	97.25±15.28	100.61±11.40	94.99±17.08	2.64	**<0.01**
Hip, cm (74-199)	102.18±13.97	101.76±12.59	102.46±14.87	-0.35	0.73
WHR (0.46-2)	0.96±0.13	0.99±0.08	0.93±0.16	3.25	**<0.01**
SBP, mmHg (90-200)	130.94±16.26	131.16±14.36	130.80±17.48	0.16	0.88
DBP, mmHg (40-125)	80.88±11.28	82.3±10.56	79.93±11.69	1.49	0.14
Glycemia, mg/dl (60-259)	95.87±29.58	97.07±24.62	94.98±32.89	0.41	0.98
HDL, mg/dl (24.4-90)	56.53±11.91	58.51±10.69	53.81±13.03	-2.28	**0.02**
TRG, mg/dl (35-284)	117.30±51.39	114.36±52.21	119.44±51.02	-0.56	0.58
HADS-A (0-20)	6.88±4.02	5.89±3.41	7.53± 4.27	<0.01	**<0.01**

HADS-D (0-13)	4.11±3.03	3.46±2.62	4.53± 3.22	0.01	**<0.01**
HADS-AD (0-32)	10.98±6.49	9.36±5.35	12.06± 6.96	<0.01	**<0.01**

Abbreviations' list: WHR, Waist-to-Hip Ratio; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High Density Lipoprotein Cholesterol; TRG, triglycerides; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale, Depression subscale; HADS-AD, Hospital Anxiety and Depression Scale, Anxiety and Depression total score.

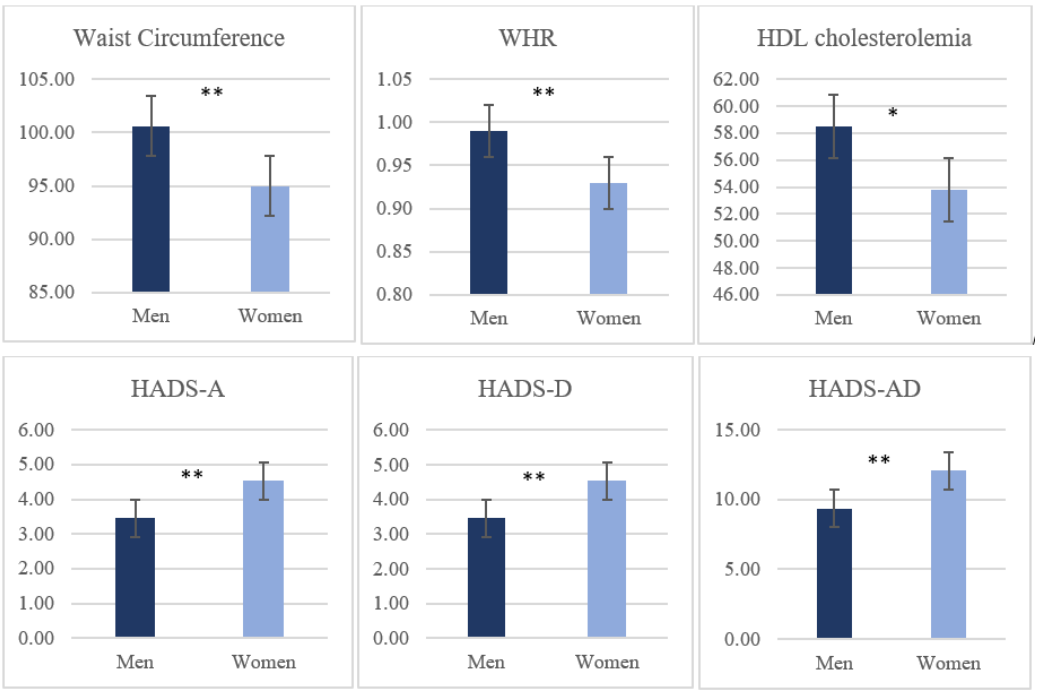


Figure 1. Statistically significant results of the gender comparison.

Abbreviations' list: WHR, Waist-to-Hip Ratio; HDL, High Density Lipoprotein Cholesterol; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale, Depression subscale; HADS-AD, Hospital Anxiety and Depression Scale, Anxiety and Depression total score; *, $p \leq 0.05$; **, $p \leq 0.001$.

The prevalence and correlations of anxiety, depression and anxious-depressive symptoms, with respect to the presence or absence of MetS (first according to ATPIII criteria – MetS-ATPIII-, then according to IDF criteria – MetS-IDF -), in both genders, are displayed in Table 3. As shown in this table, among women, the diagnosis of MetS, both according to ATPIII and to IDF criteria, significantly correlated to depressive symptomatology and to anxious-depressive symptoms.

Table 3. Prevalence and correlations of anxiety, depressive and anxious-depressive symptoms (as dichotomous variables) according to the presence or absence of MetS-ATPIII and MetS-IDF and according to gender.

	MetS + (ATP-III R)		**MetS - (ATP-III R)**		χ^2; *p*
	Women *N* (%)	Men *N* (%)	Women *N* (%)	Men *N* (%)	
HADS-A	22 (48.89)	9 (37.5)	36 (44.44)	16 (25.64)	*Women:* χ^2=0.16, p= 0.69 *Men*: χ^2=0.77, p=0.38
HADS-D	11 (24.44)	3 (10)	11 (13.58)	5 (9.26)	*Women:* χ^2=6.78, **p<0.01** *Men*: χ^2=0.00, p=0.97
HADS-AD	11 (24.44)	2 (6.67)	11 (13.58)	3 (5.56)	*Women:* χ^2=6.78, **p<0.01** *Men*: χ^2=0.22, p=0.64
	MetS + (IDF)		**MetS - (IDF)**		χ^2; *p*
	Women *N* (%)	Men *N* (%)	Women *N* (%)	Men *N* (%)	
HADS-A	20 (52.63)	8 (33.33)	16 (44.44)	8 (25.81)	*Women:* χ^2=0.50, p=0.48 *Men*: χ^2=0.37, p=0.54
HADS-D	11 (28.95)	4 (16.67)	3 (8.33)	3 (9.68)	*Women:* χ^2=5.12, **p=0.02** *Men*: χ^2=0.59, p=0.44
HADS-AD	11 (28.95)	2 (8.33)	3 (8.33)	3 (9.68)	*Women:* χ^2=5.12, **p=0.02** *Men*: χ^2=0.03, p=0.86

Table 4 reports the statistically significant results of the multivariate stepwise logistic regression analysis for symptoms of anxiety, depression and co-occurring anxious-depressive symptoms, respectively. Symptoms of anxiety were inversely associated to employment status (OR=0.28, p=0.04), and positively associated to abdominal obesity (waist circumference according to ATPIII criteria) (OR=2.07, p=0.03). Both depressive symptomatology (OR=0.17, p<0.01) and symptoms of anxiety and depression (OR=0.20, p<0.01) were found to be inversely associated to high education level.

Table 4. Results of the stepwise multivariate regression analysis for symptoms of anxiety, depression and comorbid anxious-depressive symptoms.

	OR	**SE**	**z**	**p**	**95%CI**
HADS-A	*Number of observations: 210; Adjusted R^2:0.10*				
Work condition	0.28	0.62	-2.05	**0.04**	-2.47; -0.06
Waist circumference- ATPIII criteria	2.07	0.33	2.19	**0.03**	0.08-1.38
HADS-D	*Number of observations: 210; Adjusted R^2:0.17*				
Education level	0.17	0.52	-3.43	**<0.01**	-2.79-0.76
HADS-AD	*Number of observations: 210; Adjusted R^2:0.20*				
Education level	0.20	0.52	-3.05	**<0.01**	-2.62-0.57

3.1.4 DISCUSSION

This study aimed to measure prevalence and associations between symptoms of anxiety and/or depression and MetS and/or its single components among primary care patients.

In our sample, the prevalence of MetS varied from 31.43% (ATPIII criteria) to 37.62% (IDF criteria), a result consistent with other studies conducted in industrialized Western countries[141]. MetS was significantly more prevalent in men *vs.* women, a result that agrees with some previous papers that detected the same sex-difference but that is in contrast with other studies[142].

Consistent with data highlighting that overweight and obesity prevalence rates are dramatically increasing in Italy[143], almost 40% of our sample had a BMI> 25 kg/m^2, corresponding to an overweight condition. The high prevalence of hypertension (58.37% of the sample) and hyperglycaemia (22.86%), whose frequency generally increases with increasing age[144], could be due to the high mean age of the patients enrolled in our study.

Compared to previous Italian epidemiological data on mental disorders in the community[145,146], we detected higher prevalence rate of symptoms of anxiety and depression. This result could be explained by several factors, including the use of different assessment methods (interview *vs.* self-report) and psychometric instruments (with different degrees of sensitivity and specificity) and sampling strategy. Moreover, in our study we used HADS, that is not intended to be a complete diagnostic instrument, but only a screening tool for identifying patients who need further psychiatric evaluation and assistance[147,148].

The association between waist circumference and symptoms of anxiety is consistent with previous research[135,149,150]. On the contrary, BMI was not associated with anxiety. Recently, several studies suggested that fat distribution and abdominal obesity (rather than increased weight or overall obesity *per se*) could be a major determinant of common psychiatric

symptoms[151,152]. Our results are in line with this perspective, highlighting the role of abdominal visceral fat, more pathogenic than subcutaneous fat on metabolic risk profiles[153], and fat distribution (central adiposity vs general obesity) are differentially associated with psychiatric symptoms[154,155].

At the multiple regression analysis, the education level emerged as a risk factor for depressive and anxious-depressive symptoms, according with our preliminary results on a smaller sample previously published[135]. This finding is in line with a large amount of literature suggesting the association between low education attainment and a higher risk of symptoms of depression and anxiety[156] in adults. According to literary data, adult socioeconomic position[156] and cultural capital[157] could eventually mediate these associations.

Finally, the multivariate regression analysis found that that to have a job was a protective factor for anxiety symptoms. While unemployment is a well-recognized risk factor for mental health problems, including depression and anxiety[158], previous studies on the comorbidity between mental disorders and employment produced mixed findings. High job demands and stress in the workplace are associated with a high risk of depression and anxiety[159,160]. However, the economic consequences of the 2008 economic crisis in Italy and of the recent COVID-19 pandemic have shown that also job loss is associated with a worsening of mental health symptoms. Our results support the findings that common mental symptoms, including anxiety, are less prevalent among the employed than among unemployed and economically inactive people[161,162]. One possible explanation is that employment is a relevant feature of personal and social identity, providing individual senses of usefulness, social integration and financial security, all factors positively related to physical and mental well-being[163].

Several limitations of this study must be acknowledged. First of all, the cross-sectional design of the present research does not allow to draw causal connections between the collected variables. Secondly, this study did not include the assessment of dietary patterns and sedentary lifestyle, possible confounders or mediators of the investigated associations. Finally, the small sample size limits the generalizability of our findings.

3.1.5 CONCLUSION

From a descriptive point of view, the photograph that can be taken from this study confirms how anxious-depressive spectrum is, in terms of prevalence, "epidemic" in the context of general medicine. This conclusion is in line with the WHO estimate, which foreshadows that mental health problems, especially depression, will represent the leading cause of mortality and morbidity by 2030[164].

Our results in terms of prevalence suggest that the ability to manage symptoms of anxiety and depression is now a skill to be acquired by general practitioners, and also a priority to be taken at the level of the re-organisation of the entire public health system.

The finding of an association between waist circumference and anxiety suggests that psychosocial and psychological treatments should be added to lifestyle changes in patients with abdominal adiposity.

The role of education and employment as a protective factor for mental health fits in the perspective of Engel's bio-psycho-social model. Strengthening institutions of training and education of the population, especially women, could be crucial and source of long-term economic benefits; future research could focus on identifying effective strategies to raise awareness among government organizations on these issues.

This research also suggests new interesting research field for the next future. It may be appropriate to examine whether anxious-depressive symptoms may give an additional medical risk, compared to cardiovascular risks, among patients suffering from MetS, overweight and obesity.

3.2 STUDY II

Preliminary results of a multidisciplinary Italian study adopting a Psycho-Neuro-Endocrine-Immunological (PNEI) approach to the study of colorectal adenomas.

These data have been already published in the following paper:
Mancini Stefano, Alboni Silvia, Mattei Giorgio, **Rioli Giulia**, Sena Paola, Marchi Mattia, Sacchetti Andrea, Boarino Valentina, Roncucci Luca, Galeazzi Gian Maria, Ferrari Silvia. Preliminary results of a multidisciplinary Italian study adopting a Psycho-Neuro-Endocrine-Immunological (PNEI) approach to the study of colorectal adenomas. Acta Biomedica 2021; Vol. 92, N.1: e2021014; 1-10. DOI: 10.23750/abm.v92i1.10197.

ABSTRACT

Background and aim: Colorectal mucosal precancerous lesions, metabolic syndrome (MetS) and psychiatric disorders may share a common low-grade local and systemic inflammation. Aim is to report on preliminary data concerning a research adopting a psycho-neuro-endocrine-immune (PNEI) approach to study outpatients undergoing colonoscopy.

Methods: A sample of patients undergoing colonoscopy was cross-sectionally investigated. Data on colorectal adenomas, MetS, early atherosclerosis, anxious-depressive symptoms, personality traits, and inflammatory markers were statistically analysed.

Results: Sixty-two patients were recruited (female 50%, mean age: 60.8±9.4 years). The prevalence of adenomas and MetS was respectively of 45.2% and 41.9%. Anxiety and depressive symptoms were detected in 16 (32.7%) and 9 (18.4%) subjects, respectively. The presence of adenomas positively correlated with male sex ($p=0.01$), age ($p<0.01$), IL-6 ($p=0.03$), CRP ($p=0.04$), and MetS ($p=0.03$); it was also associated with CRP concentration (OR=3.81, $p=0.03$).

Conclusions: Proinflammatory atherogenic status, psychological traits, increased mucosal inflammation, and metabolic parameters may share a common a pathogenic mechanism, worth studying.

3.2.1 INTRODUCTION

A major cause of disability and mortality in Western countries is represented by colorectal cancer (CRC). CRC develops from benign precursors that transform into cancer in a stepwise manner; several molecular abnormalities accompany determine colorectal tumorigenesis[165]. The risk of CRC is increased in patients affected by Metabolic Syndrome (MetS), a cluster of cardiovascular risk factors affecting about a quarter of adult population worldly, clinically defined as a combination of visceral obesity, increased blood pressure, insulin resistance and dyslipidaemia[166]. MetS components seem to share a common underlying pathophysiology that

may also contribute to the progression of atherosclerosis[167]. MetS frequency is higher in Western countries and among people aged 40-80, and its prognostic implications are considerable: people with MetS have a doubled risk of dying of cardiovascular diseases and a tripled risk to suffer from heart attack or stroke, as well as all-cause mortality[168]. Anxiety and depressive disorders are common among patients diagnosed with cancer, as well as among patients affected by MetS[135,169]. Such disorders are related to extreme distress, low self-perceived health-related quality of life and significant economic costs. Moreover, according to recent studies, personality traits could be considered psychological risk factors for CRC, in addition to environmental and social risk factors[128]. Psychological traits may also contribute to MetS, suggesting a mediation by reciprocal risk factors, both behavioural such as eating habits, physical inactivity and smoking, and biological such as inflammation and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis[170]. Elevated levels of myeloperoxidase (MPO), a member of the superfamily of heme-peroxidases, are associated with inflammation and increased oxidative stress. Evidence suggests that MPO could be associated with increased risk of cardiovascular diseases and might be a marker of colorectal cancer risk in normal colorectal mucosa[171]. In the light of the above, the psycho-neuro-endocrine-immune (PNEI) perspective could represent a useful and feasible model to understand the complex bio-psycho-social comorbidities between CRC, MetS, anxious-depressive symptoms, personality traits and inflammation. Therefore, aim of the present study was to describe a sample of outpatients undergoing colonoscopy, by adopting a PNEI perspective, to assess whether such an integrated approach could help understand the complex cross-talk between different risk factors and states of health and disease, driving the multi-assessment of patients in the clinical practice. In particular, we aimed to assess the coexistence of multidistrict low-grade inflammation with metabolic and psychological conditions.

3.2.2 MATERIALS AND METHODS

Study design and sample

Cross-sectional study. Data were collected from 1 January 2015 to 30 June 2016. Patients were enrolled from a population referred to colonoscopy because of either a history or new evidence suspicious for colorectal neoplastic disease (e.g. positive faecal occult blood), or non-specific abdominal symptoms.

Statement of ethics

The study was approved by the competent Ethic Committee and the Local Health Agency of Modena. Every patient enrolled in the study signed a detailed written informed consent. The study was carried out according to the Declaration of Helsinki, to the Good Clinical Practice principles for medical research and to the current regulations relating to the protection and processing of personal and sensitive data (European Regulation n. 679/2016).

Data collection

For each patient, demographic (age, gender) and anthropometric characteristics (height, waist and hip circumference, in centimetres; body weight, in kilograms) were collected. BMI (body weight in kilograms/height in metres^2) and waist-to-hip ratio were then calculated manually. Systolic and diastolic arterial blood pressure (mmHg) were measured by means of a manual sphygmomanometer at the right arm. A detailed medical history was obtained for each patient, including on-going medications (psychotropic medication: antidepressant and/or anxiolytic drug therapy; cholesterol-lowering medications: statins). Dichotomous data (yes/no) on alcohol consumption, smoking habit and sedentary lifestyle were also collected. Finally, the Cumulative Illness Rating Scale (CIRS) was calculated for each patient to assess multimorbidity. Results were then collected of the following assessments and laboratory analyses:

1) Immunofluorescence by confocal microscopy on colonic biopsies

Three samples of normal colorectal mucosa (NM) were collected during colonoscopy (right colon, descending colon, and sigmoid colon-rectum). The samples of NM collected, were fixed in 3% formalin and embedded in paraffin for immunofluorescence analysis. The immunofluorescence experiments were performed as previously described[172]. To each sample was assigned a code number and the score, referred to as Immuno-Fluorescence Intensity Score (IFIS), was determined by an observer who was blind to tissue groups during the analysis[172]. Data were expressed as a mean of the IFIS in the three segments of the intestine collected for each patient as previously described[173]. Blood samples were drawn before performing colonoscopy, immediately processed for serum extraction by centrifugation and stored frozen at -80 °C until test execution. The following biochemical analyses were performed: high sensitive (hs) CRP (mg/L); glycaemia (mg/dL), total cholesterol (mg/dL), low-density lipoprotein (LDL) cholesterol (mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), very low-density lipoprotein (VLDL) cholesterol (mg/dL), triglycerides (mg/dl). Serological analyses were performed on a platform validated for clinical purposes (Roche Diagnostics S.p.A., Cobas® System, Monza, Italy). Serological and immunohistochemistry tests were performed in blind for anthropometric data and other clinical records.

2) Enzyme Linked ImmunoSorbent Assays (ELISA)

A highly sensitive quantitative sandwich enzyme immunoassay technique, Quantikine® HS ELISA KIT (R& D systems, Minneapolis, USA), was used to measure circulating serum levels of IL-6 in accordance with the manufacture's instruction starting from 200 μL of diluted sera or standard solutions. After adding Stop Solution to each well and the optical density was measured by using a Thermo Scientific Multiskan® FC microplate reader, at 490 and 690 nm. IL-1 levels were measured by pre-coated ELISA using Boster Human IL-1 Picokine TM

ELISA KIT according to the manufacturer's instruction staring from 100 µL of standard solutions, samples (dil. 1:3) or control. The reaction was stopped by adding 100 µL of Stop Solution and O.D. absorbance read by using the microplate reader, at 450 nm. IL-1 was decantable in 18 out of 62 patients. TNF-and IFN-levels were also measured in the serum of patients by using comedically available highly sensitive Picokine ELISA KITs, but the cytokine levels were under the limit of detection of these KITs.

3) Ultrasound vascular evaluation

To measure early atherosclerosis, an ultrasound technique of intima-media thickening measurement of the common carotid artery wall (qIMT, carotid intima-media thickness) was used. Enrolled patients underwent ultrasound examination on an Esaote MyLab25 platform (Esaote Medical Systems, Italy) using a high-resolution 7–13 MHz linear-array transducer (LA523) by an experienced angiographer. The system employed dedicated software RF-tracking technology to obtain RF Quality IMT (RF-QIMT). The sonographer was blinded to the patients' results of the other tests performed during this study. Image acquisition included the evaluation of the right and left common carotid arteries (LS-qIMT, left side carotid intima-media thickness; RS-qIMT, right side carotid intima-media thickness), 1 cm proximal to the carotid bulb. All measurements were taken in the supine position with patient at rest and comfortable. The thickness of the intima-media layer was expressed in micrometer with a minimum resolution of 12 micrometers, and the result accepted when the standard deviation of the multiple automated acquisitions was within 20 micrometers. To measure endothelial function, we used flow-mediated dilation technique executed on the brachial artery in the non-dominant arm. Brachial flow-mediated dilation (brachial FMD) was performed according to the literature[174]. Brachial artery diameter measurements were performed on a segment of about 2 cm in length. These were performed at baseline, 60 seconds after hyperaemia, and 3 minutes after hyperaemia. The hyperaemia (endothelium-dependent dilation) is induced by the inflation of an adult cuff of a common sphygmomanometer for blood pressure measurement. The cuff is positioned in the proximal portion of the arm to stop the flow of the artery and it is kept for 5 minutes. Brachial artery diameter was expressed in millimeters with two decimals. The flow mediated dilation (FMD) is defined as the maximum percentage increase in the artery diameter during hyperaemia following the release of the pressure in the cuff, and the subsequent reperfusion of the artery.

4) Psychometric assessment

Patients were asked to fill in the following 4 self-administered psychometric instruments:

a) *The Hospital Anxiety and Depression Scale (HADS), Italian version:* rating scale made of 7 questions for anxiety and 7 for depression, developed to assess the presence and severity of depressive and/or anxious symptomatology in the week before administration. Although originally designed to be used with hospital populations, it has been found to be a valid and

reliable instrument among medical and/ or psychiatric outpatients and for screening procedures; clinically-significant symptoms of anxiety and/or depression are suggested by a score of 8 or more on each subscale;

b) *The Temperament and Character Inventory (TCI), Italian version:* 240-items test for personality traits to assess the intensity of and the relationships between the 4 basic dimensions of temperament (Novelty Seeking (NS), Harm Avoidance (HA), Reward Dependence (RD) and Persistence (P)) and the 3 dimensions of character (Self-Directedness (SD), Cooperativeness (C) and Self-Transcendence (ST)). It is based on a psychobiological model that attempts to describe and explain the causes of individual differences in personality traits;

c) *The INTERMED (INTERdisciplinary MEDicine) Self-Assessment (INTERMED):* it is a recently developed tool, validated in the Italian language, consisting in 27 multiple-choice questions and aiming at measuring bio-psycho-social complexity impacting on organization of care;

d) *The 36-item Short Form Survey (SF36), Italian version:* assess patients' perception of own general health status, consisting in 8 scaled scores, each exploring one of the following domains: vitality, physical functioning, bodily pain, general health perceptions, mental health, physical role functioning, emotional role functioning, social role functioning.

Statistics

All data were recorded anonymously in a Microsoft Excel sheet and further analysed by means of STATA 13.1 (College Station, TX, USA). Descriptive statistics were performed by means of means, frequencies, standard deviations and ranges. Correlation analyses between variables were performed by means of Pearson's coefficient (r). To reduce the risk of type I error, q-value throughout Holm's correction were calculated. Level of statistical significance was set at alpha = 0.05. Finally, simple and multiple binary logistic regressions models were run. The outcome was represented by a binary variable, equal to 1 when at least one adenoma was detected at the colonoscopy, 0 otherwise. Explanatory variables were all those collected from anamnesis and physical examination, colonoscopy, immunofluorescence by confocal microscopy on colonic biopsies, ELISA analysis, ultrasound vascular evaluation, and psychometric assessment. A stepwise multiple regression analysis was run, including all those variables that had reached a $P < .25$ level of statistical significance (the latter set to reduce type II error). Differently, in the multivariate regression analysis the usual level of significance ($P < .05$) was set.

3.2.3 RESULTS

Descriptive analysis

Sixty-two patients were recruited (31 women, 50%, and 31 men, 50%) with a mean age of 60.8±9.4 years. Table 1 displays the absolute (on the left) and gender-related (on the right) prevalence in the sample of risk factors and/or diseases, as dichotomous variables.

Table 1. General features of the sample (dichotomous variables).

Dichotomous Variable	**Number**	**%**	**Missing**
Sex (Men/Women)	31/31	50/50	0
Smoke (Yes/No)	22/40	35.5/64.5	0
Alcohol (Yes/No)	42/20	67.7/32.3	0
Sedentary (Yes/No)	25/37	40.3/59.7	0
Adenoma (Yes/No)	28/34	45.2/54.8	0
MetS (Yes/No)	26/36	41.9/58.1	0
Antidepressants (Yes/No)	3/59	4.8/95.2	0
Anxiolytics (Yes/No)	4/58	6.5/93.5	0
Statins (Yes/No)	15/47	24.2/75.8	0
HADS-A (positive/negative)	16/33	32.7/67.3	13
HADS-D (positive/negative)	9/40	18.4/81.6	13
HADS-AD (positive/negative)	4/45	8.2/91.8	13
INTERMED (positive/negative)	0/28	0/100	34
Variable	**Men/Women (%)**		
Presence of Adenomas	19/9(67.9/32.1)		
Absence of Adenomas	13/22(39.4/71.0)		
MetS positive	15/11(57.7/42.3)		
MetS negative	16/20(44.4/55.6)		

Twenty-two subjects (35.5%) were current smokers, 42 (67.7%) declared to generally drink alcoholic beverages and 25 (40.3%) admitted having a sedentary lifestyle. Only 3 patients

(4.8%) currently used antidepressants and 4 (6.5%) anxiolytic medications; 15 patients (24.2%) currently used statins. Twenty-eight participants were affected by at least one adenoma (45.2% of the total sample); in this group, 19 (67.9%) were men. MetS was diagnosed in 26 participants (41.9% of the sample, according to the ATP-III-R criteria). Clinically significant symptoms of anxiety were reported by 16 patients (32.7%); 9 individuals reported the presence of depressive symptoms (18.4%), and 4 presented combined anxiety-depressive symptoms (8.2%). Figure 1 displays the prevalence and comorbidity of adenomas, MetS and symptoms of anxiety and/ or depression, among the 49 participants for whom all variables were collected and available. The intersections between the closed curves show the areas of comorbidity between different diseases. In particular, 1 patient was simultaneously affected by all these conditions (adenoma, MetS, symptoms of anxiety and depression); 3 patients by both anxiety and adenoma; 1 patient by adenoma and comorbid anxious-depressive symptomatology; 2 patients by adenoma, MetS and symptoms of anxiety; and 1 patient by adenoma, MetS and depression.

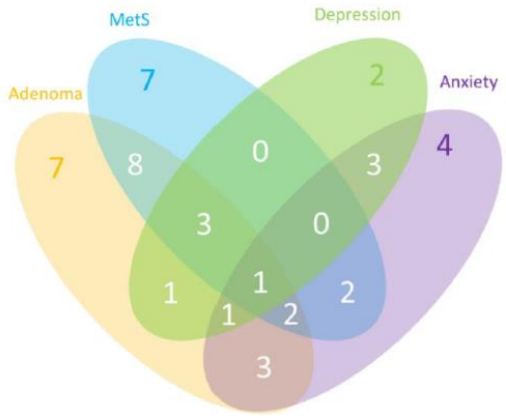


Figure 1. Venn Diagram. Prevalence of adenoma, Metabolic Syndrome, symptoms of anxiety and symptoms of depression, and their comorbidity, among 49 patients of the sample.

Demographic, anthropometric, and clinical characteristics of the patients, as continuous variables, are displayed in Table 2.

Table 2. General features of the sample (continuous variables).

Continuous Variable	**N**	**Missing**	**Mean**	**SD**	**Max**	**Min**
Age, years	62	0	60.8	9.4	82	44
BMI, kg/m^2	62	0	27.3	4.6	38.1	18.8
WHR	62	0	0.9	0.1	1.2	0.8
SBP, mmHg	62	0	146	18.2	180	110

DBP, mmHg	62	0	83.2	10.3	105	60
Glycemia, mg/dl	62	0	94.1	18.3	194	64
Total Cholesterol, mg/dl	62	0	196.2	19.2	241	156
HDL	61	1	49.9	10.8	77	32
LDL	61	1	119.9	16.7	154	84
VLDL	62	0	26.6	9.1	44	11
TRG	62	0	132.7	45.3	220	57
RS-qIMT	57	5	817.8	286.4	1500	434
LS-qIMT	57	5	841.2	305	2000	503
FMD basal-1 min%	56	6	9.6	5.6	21.1	0
FMD basal-3 min%	56	6	8.6	5.9	20	-7.3
FMD 1 min-3 min%	56	6	-1.3	6.2	15.5	-22.5
HADS-A	49	13	5.7	3.9	15	0
HADS-D	49	13	4.4	3.1	11	0
TCI-NS	43	19	22	3.8	29	15
TCI-HA	43	19	18.4	2.9	24	10
TCI-RD	43	19	11.1	2.7	18	6
TCI-P	43	19	5.4	1.6	8	3
TCI-SD	43	19	26.4	3.9	34	18
TCI-C	43	19	21.9	4.6	31	12
TCI-ST	43	19	20.4	3.5	28	12
SF36-PCS	49	13	47.5	7.9	58.8	26.1
SF36-MCS	49	13	51.3	8.8	66.1	22.8
SF36-CS	49	13	2.8	0.9	1	4
INTERMED	28	34	7.8	4.2	3	18
CRP, mg/L	62	0	0.6	0.4	2.1	0.2
IL-6, pg/ml	49	13	0.8	0.8	5	0.2
IL-1, pg/ml	18	44	6.0	9.3	41.7	0.8
MPO mean	62	0	60.9	8.9	80.7	37.3
CIRS	62	0	0.2	0.1	0.5	0

As to cardiometabolic parameters, the mean BMI of the sample was 27.3 kg/m^2 (SD=4.6 kg/m^2); mean Systolic Blood Pressure was 146.0 mmHg (SD=18.2 mmHg), mean Diastolic Blood Pressure was 82.2 mmHg (SD=10.3 mmHg). The mean level of blood glycaemia was 94.1 mg/dl (SD=18.3 mmHg), mean total cholesterol was 196.2 mg/dl (SD=19.2 mg/dl). RS-qIMT was 817.8 micrometers (SD=286.4 micrometers), while LS-qIMT was 841.2

micrometers (SD=305.0 micrometers). As to results of psychometric tests, data are less complete (13 missing data for HADS and SF36; 19 for TCI; 34 for INTERMED). Mean scores at the HADS were 5.7±3.9 for anxiety and 4.4±3.1 for depression. TCI and SF36 scores both demonstrate a complete range of different psychological profiles and conditions (different dimensions at TCI), as indicated by the mean values as well as by standard deviations and ranges. Finally, as to parameters of inflammation, the CRP mean value was 0.6 mg/dL, indicating a generally low level of inflammation. While TNF-and INF-levels were undetectable in the serum of patients, IL-1 mean level (available for 18 subjects) was 6.0 pg/ mL in 18 out of 62 patients and IL-6 mean level was 0.8 pg/mL (available for 49 subjects). MPO mean value throughout colorectal mucosa was 60.9 (SD=8.9, available for 62 subjects).

Correlation analysis

The presence of adenomas was associated with male sex ($r=0.32$; $p=0.01$), age ($r=0.34$, $p<0.01$), IL-6 ($r=0.31$; $p=0.03$), CRP ($r=0.27$; $p=0.04$) and diagnosis of MetS ($r=0.28$; $p=0.03$). MetS was associated with age ($r=0.32$; $p<0.001$), IL-6 ($r=0.37$; $p<0.01$), CRP ($r=0.54$; $p=0.00$), presence of adenomas ($r=0.27$; $p=0.03$) and CIRS ($r=0.56$; $p=0.00$). Both HADS-A ($r=-0.65$; $p=0.00$) and HADS-D ($r=-0.45$, $p<0.01$) scores were associated with SF36 MCS; HADS-D was also associated with SF36 PCS ($r=-0.39$, $p<0.01$). An ongoing antidepressant therapy was associated with diastolic blood pressure ($r=0.26$, $p=0.04$), glycemia ($r=0.38$, $p<0.01$) and RS-qIMT ($r=0.37$, $p<0.01$). Various inter-correlations related to temperament dimensions as measured by the TCI were also found. Novelty Seeking was associated with Harm Avoidance ($r=0.40$, $p<0.01$) and Self-Transcendence ($r=0.34$, $p=0.03$); Self-Directedness was associated with Cooperativeness ($r= 0.63$, $p<0.01$) and Self-Transcendence ($r=0.55$, $p<0.01$); Cooperativeness was associated with Self-Transcendence ($r=0.62$, $p<0.01$). Novelty Seeking was associated with right qIMT ($r=-0.31$, $p=0.04$); Harm Avoidance was associated with right qIMT ($r=-0.56$, $p<0.01$); Reward Dependence was associated with weight ($r=-0.51$, $p<0.05$), height ($r=-0.55$, $p<0.01$), BMI ($r=-0.35$, $p=0.02$), waist circumference ($r=-0.36$, $p=0.02$) and presence of medical comorbidities (CIRS score) ($r=0.38$, $p=0.01$); Persistence was associated with weight ($r=0.35$, $p=0.03$) and height ($r=-0.34$, $p=0.03$); Cooperativeness was associated with statin therapy ($r=-0.31$, $p=0.03$). Novelty Seeking was associated with SF36 MCS ($r=-0.43$, $p<0.05$), Harm Avoidance was associated with SF36 PCS ($r=0.30$, $p<0.05$), and finally Cooperativeness was associated with SF36 MCS ($r=-0.31$, $p<0.05$). In addition to the observed association with the presence of adenomas and the diagnosis of MetS, CRP resulted directly related to cholesterol values (total cholesterol: $r= 0.42$; $p<0.01$; LDL cholesterol: $r=0.32$; $p= 0.01$; VLDL cholesterol: $r=0.26$; $p= 0.04$), triglycerides ($r= 0.27$; $p= 0.04$), BMI ($r=0.38$, $p<0.01$), weight ($r=0.39$; $p<0.01$), waist ($r=0.46$, $p<0.01$) and hip circumferences ($r=0.33$; $p<0.01$) and to waist-to-hip ratio ($r=0.39$, $p<0.01$).

Moreover, CRP was related to both diastolic (r=0.29, p=0.02) and systolic blood pressure (r=0.29, p=0.02), sedentary lifestyle (r=0.26, p=0.04) and CIRS (r=0.47, p<0.01). A few correlations were detectable also with respect to results at the ultrasound vascular examination (IMT): RS-qIMT and LS-qIMT resulted to directly covariate (r=0.33, p<0.01), though in few cases different correlations have been found between a variable and RS-qIMT or LS-qIMT. Moreover, both right and left side qIMT resulted directly related to cholesterol values and particularly to LDL cholesterol (RS-qIMT: r=0.40, p<0.01; LC-IMT: r=0.37, p<0.01). qIMT resulted to be directly related to BMI, weight, glycaemic values, and MetS. Higher values of qIMT were significantly associated with the presence of one or more adenomas in the colonic tract (RS-qIMT: r=0.30, p=0.03; LS-qIMT: r=0.33, p=0.01). Mean MPO and LS-IMT were inversely related (r=-0.28; p=0.03). After Holm's correction, the following correlations remained significant: sex - BMI (q-value: 0.04); age - TCI-HA (q-value: 0.05); age-smoke (q-value: 0.003); MetS - WHR (q-value: 0.05); MetS - SBP (q-value: 0.03); MetS - glycemia (q-value: 0.02); HADSD - SF36MCS (q-value: 0.04); TCIHA -RS-qIMT (q-value: 0.003); TCI-SD- TCI-ST (q-value: 0.007); SF36-PCS - sedentary lifestyle (q-value: 0.007); BMI - SBP (q-value: 0.01); BMI - DBP (q-value: 0.04); BMI - CIRS (q-value: 0.009); WHR - DBP (q-value: 0.03); WHR - CRP (q-value: 0.05); FMD1min - FMD1-3min (q-value: 0.01); FMD3min-FMD1-3min (q-value: 0.006); CIRS - CRP (q-value: 0.003).

Binary logistic regression analysis

Table 3 shows the variables that had reached a p <0.25 level of statistical significance at the simple regression analysis. The stepwise regression analysis carried out on the list of variables reported in Table 3 pointed out that only increased cholesterol levels were associated with increased presence of colorectal adenomas (OR=1.07, p<0.01, 95%CI=1.02; 1.10). Notably, a 10% significance level was reported by the association between LS-qIMT and outcome (OR= 1.00, p=0.09, 95%CI=1.00-1.04).

Table 3. Variables included in the stepwise logistic regression (observations: 37; pseudo-R squared: 0.32).

Variable	**Odds Ratio**	**P-value**	**95% CI**
Diagnosis of MetS	0.13	0.16	0.01; 2.17
Cholesterol levels	1.06	0.05	9.99; 1.13
LS-qIMT	1.00	0.10	0.99; 1.01
CIRS	0.90	0.92	0.12; 6.92

HADS-D	0.97	0.87	0.67; 1.41
SF36-MCS	1.05	0.48	0.92; 1.20
CRP	6.96	0.15	0.50; 96.53
IL-6	1.30	0.88	0.05; 35.68

3.2.4 DISCUSSION AND CONCLUSIONS

The sample enrolled in the present study was consistent with the adult western population undergoing first-line diagnostic procedures: a quarter of the sample were currently following a statin prescription, suggesting that, despite an average level of cholesterol of almost 200 mg/dl, hypercholesterolemia was rather common, in line with Italian prevalence data[175]. Psychological symptoms were generally low, as expected in a non-selected population, with mean scores at the HADS largely below the cut-off of clinical significance. MetS was suggested to be related to several of the other measures collected, and specifically: circulating levels of IL-6 and CRP, a well-known marker of systemic inflammation; carotid IMT, a relevant indicator of early atherosclerosis and powerful predictor of coronary artery disease and stroke[171]; score at the CIRS, a significant proxy of bio-psycho-social complexity; and, finally, presence of at least one adenoma in the large bowel. Adenoma is a well-known step of the adenoma-carcinoma sequence. Epidemiological studies have demonstrated that colorectal adenomas and CRC risk are higher in individuals with MetS or any of its components. A recent meta-analysis of cohort studies reported that MetS was associated with higher relative risks of CRC both in men and in women[176]. The association of MetS and obesity with intestinal adenomas was already observed in larger populations[168], suggesting that MetS could be considered in risk stratification for surveillance intervals for colonoscopy.

Moreover, an interesting result is the significant correlation of CRP and IL-6 with both the presence of colorectal adenomas and MetS, suggesting that even a low grade of inflammation may be a useful indicator of those common conditions.

MetS and psycho-social issues may be associated either directly, throughout shared biological pathways, or indirectly. In fact, personality traits widely affect behaviour, leading individuals to have various levels of attention of good health and positive lifestyle, as well as different attitudes toward screening procedures[177]. Health behaviours may also be affected by socio-economic conditions and environmental factors[178]. The presence of anxiety and depressive symptoms was associated with a generally worse health status, consistently with previous research[179]. Intra-correlations between TCI dimensions were only small, but in line with previous data mainly supporting Cloninger's theory of independent dimensions[98]. In contrast with previous findings, we could not replicate the presence of significant associations between

personality traits and anxiety-depressive symptomatology, as initially hypothesised, though this could be due to methodological problems or sample size dimensions. The negative association between Cooperativeness and ongoing therapy with statins, even if with moderate effect size, is consistent with other findings in literature. Indeed, this dimension of personality relates to facets of agreeableness/hostility, and it was hypothesised that people with higher levels of hostility are more likely to have more cardiovascular risk factors, such as dyslipidaemia, hypertension, obesity and even MetS[98]. It could be argued that this association is mediated by both behavioural factors, such as diet and smoking, and biological factors, namely HPA dysregulation, that leads angry and hostile individuals to have chronically higher levels of cortisol, cardiovascular and neuroendocrine reactivity[170].

This study has several limitations that need to be acknowledged. First, the cross-sectional observational nature of the study does not allow to establish a causal or etiological association between the collected variables. Second, no control group was included. Third, the sample size was small, and further limited by the fact that not every patient completed the psychometric questionnaires. Such features limit the generalizability findings; nevertheless, the sample was enrolled from outpatients performing screening procedures, thus minimizing selection bias from highly selected populations. Fourth, the psychopathological assessment was performed exclusively by means of self-reported questionnaires, limiting extent, reliability and depth of collected data; anyway, the tools were all validated and commonly used for similar aims. Despite all limitations, the present study represents a first approach to implement at the clinical and research level a multi-disciplinary, PNEI approach adopted, increasingly accepted as a validated paradigm in the scientific community. Further studies, with larger sample sizes and adopting a prospective design are currently on our research agenda, to improve the limitations of this research and provide more evidence able to sustain this approach.

In conclusion, this study suggested the usefulness of implementing a clinical multidimensional model in a PNEI perspective; such approach provided hints of evidence concerning connections among a proinflammatory atherogenic status, some psychological traits, increased mucosal inflammation and metabolic parameters in a sample of outpatients screened for colonic adenomas. Also, the findings of this study provide a preliminary support that colonic adenomas might arise from complex and interconnected clinical and psychopathological substrates in humans.

3.3 STUDY III

Gender Differences in Anxious-Depressive Symptomatology, Metabolic Syndrome and Colorectal Adenomas among outpatients undergoing colonoscopy: a cross-sectional study according to a PNEI perspective.

The results of this study have been already published in the following paper:
Rioli Giulia, Mattei Giorgio, Bonamici Caterina, Mancini Stefano, Alboni Silvia, Canazza Giuseppe, Sena Paola, Roncucci Luca, Pingani Luca, Ferrari Silvia, Galeazzi Gian Maria. Gender Differences in Anxious-Depressive Symptomatology, Metabolic Syndrome and Colorectal Adenomas among outpatients undergoing colonoscopy: a cross-sectional study according to a PNEI Perspective. Acta Biomedica Atenei Parmensis 2022, 93(4): e2022258. DOI: 10.23750/abm.v93i4.12463.

ABSTRACT

Aim: To explore gender differences in patients suffering from anxious-depressive symptoms, Metabolic Syndrome (MetS) and Colorectal Adenomas (CRAs) among a sample of outpatients undergoing colonoscopy for screening purposes.

Methods: Cross-sectional study. 126 consecutive outpatients of both sexes undergoing colonoscopy for non-specific abdominal symptoms between January 2015 and June 2021 at the Modena Policlinico General Hospital (Modena, Northern Italy) were enrolled. MetS was diagnosed according to ATPIII and IDF criteria. Anxiety and depression were assessed with the Hospital Anxiety and Depression Scale (HADS), while the Temperament and Character Inventory (TCI) was used to study personality. The SF-36 was also included as a measure of quality of life perception.

Results: Among 126 outpatients (51.60% male) undergoing colonoscopy, 51 (44%) had CRAs, 54 (47%) MetS, 41 (41.40%) anxiety symptoms, 22 (22.20%) depressive symptoms and 13 (13.10%) combined anxious-depressive symptoms. HADS-A ($t=2.68$, $p=0.01$) and TCI Reward Dependence (TCI-RD) ($t=3.01$, $p=0.00$) mean scores were significantly higher in women; conversely, SF-36 Mental Component Summary scores were higher in men. CRAs were significantly prevalent in men ($\chi^2=9.32$, $p=0.00$) and were statistically significantly associated with male sex at the univariate logistic regression analysis (OR=3.27; $p<0.01$). At the multivariate logistic regression, diastolic hypertension ($p<0.01$) was positively associated with male sex, while TCI-RD ($p=0.04$) and HDL hypocholesterolemia ($p=0.02$) were inversely associated with male sex.

Conclusions: Several significant gender differences in anxious-depressive symptoms, MetS and CRAs were found. These preliminary data suggest the need to consider gender specificities while implementing therapeutic, diagnostic, and preventive strategies.

3.3.1 INTRODUCTION

In Europe, an estimated 165 million people, over 38% of the adult population, suffer from a mental disorder each year, with depression and anxiety being among the most common mental health issues[50]. These disorders account for up to 40% of years lived with disability, with depression as the main cause[41].

Stress-related disorders such as anxiety and depression are extremely prevalent in women[180]. Subclinical anxiety and depression symptoms are also more prevalent in women[181].

Several papers have investigated the biological and cultural reasons accountable to the gender gap in the epidemiology and pathophysiology of affective and common emotional disorders[182]. First of all, sex differences in brain structure and function could be relevant[183-188]. Moreover, the fluctuations in gonadal and stress hormones across the menstrual cycle and during major lifetime hormonal influences (i.e., puberty, pregnancy, lactation, menopause) can play a major role in predisposing females to stress-related diseases[180,182,189]. Sex differences in the inflammatory response and the inflammation-induced kynurenine pathway (KP) may also be involved[190]. Gender related differences in cognitive processes[191,192] are thought to result in women experiencing more sensitivity to interpersonal stressors, rejection, criticism and separation, key features of depression and anxiety disorders[193-195]. Social determinants including gender stereotypes and roles, economical inequalities[196] and exposure to domestic or sexual violence[197], interacting with biological factors, finally, are generally known to contribute to female vulnerability to mental health disorders[198].

In clinical practice, both anxiety and depressive symptoms are frequently comorbid with internal and chronic degenerative diseases, i.e. Metabolic Syndrome (MetS) and Colorectal Adenomas (CRAs). An emerging body of evidence has demonstrated the association between MetS and colorectal cancer (CRC)[103-105,199]. Conversely, medical literature examining the relationship between MetS and CRAs is more limited[200-202].

Gender differences have been found in the prevalence of both MetS and its diagnostic components[203-205] and CRAs[200,206]. In a recent community-based study, an effect of MetS on CRAs was observed in both genders, whereas the contribution of the individual components of MetS differed between men and women[200].

Clinical[206] and preclinical studies[207] have shown sex differences in inflammatory response in MetS, suggesting the possible different role of inflammatory processes in the pathogenesis of MetS in men and women. Several sex differences were also found in the association between C-Reactive Protein (CRP) and CRAs, suggesting that diet and lifestyle lowering inflammation

may be a strategy to prevent neoplasms[202]. Interestingly, alterations of the inflammation-induced effects on KP and its metabolites were also found both in MetS[208,209] and in CRAs patients[40,211].

Therefore, taking an integrated psycho-neuro-immuno-endocrinological (PNEI) perspective, it is reasonable to hypothesize that sex differences in circulating levels of inflammatory markers such as CRP and metabolites belonging to the KP[212,213] could eventually influence the development of anxious-depressive symptoms, MetS, CRAs and their associations, further underlining the necessity to investigate gender differences in this field.

The aim of the present study was to investigate gender differences in anxious-depressive symptomatology, MetS and CRAs, in a sample of outpatients undergoing colonoscopy, adopting an integrated PNEI perspective.

3.3.2 MATERIAL AND METHODS

Ethics

All patients enrolled in the study provided written informed consent. The study was conducted in accordance with the Helsinki Declaration for ethical standards in medical research. The study was part of a wider project called *"Sovrappeso e infiammazione della mucosa colorettale come parametri di rischio neoplastico intestinale"* and was approved by the local Ethics Committee (Comitato Etico Provinciale di Modena, prot. No. 4396/C.E.), whose preliminary results on a limited sample has been previously published[201,128].

Study design

The study followed a cross-sectional design.

Study population

126 consecutive outpatients, of both sexes, who underwent colonoscopy between January 2015 and June 2021 for non-specific abdominal symptoms (abdominal pain, bowel movements abnormalities, or hematochezia) or occasional positive faecal occult blood, were enrolled in the study. Patients with a positive history of colorectal neoplasms, or with a history of, or ongoing systemic condition at an advanced stage, or affected by an inflammatory bowel disease were excluded from the study participation.

Data collection

Before the colonoscopy, biometric parameters were measured and collected, including: weight (kg), height (m), waist circumference (cm) and systolic (SBP) and diastolic (DBP) blood pressure (mmHg). BMI (body weight in kg/height in m^2) and waist-to-hip ratio (WHR) were calculated. Patients were also interviewed regarding their smoking status, alcohol consumption and level of physical activity. A past medical and psychopharmacological history was detailed for each patient.

Before undergoing the colonoscopy, a 10-mL venous blood sample for serological analysis was collected from patients and immediately processed for hs-CRP (mg/L), glycaemia (mg/dl) and lipid profile (triglycerides, TRG, mg/dl; total cholesterol, mg/dl; low-density lipoprotein cholesterol, LDL, mg/dl; high-density lipoprotein cholesterol, HDL, mg/dl).

After the colonoscopy, each patient was asked to fulfil the following self-administered tests for the psychometric assessment. Each test was used in its validated Italian-language version.

a) *Hospital Anxiety and Depression Scale, HADS*. It is a 14-items self-rating scale developed to assess the presence and the severity of anxious and depressive symptoms both in hospital populations and in community settings. It is composed of 7 questions for anxiety and 7 questions for depression[138,139,214], therefore providing both an anxiety (HADS-A) and a depressive (HADS-D) score.

b) The *Temperament and Character Inventory (TCI)*. It is a 240-items self-report test for personality assessment, aimed to evaluate the main temperament (novelty seeking, NS; harm avoidance, HA; reward dependence, RD; persistence, P) and character traits (self-directedness, SD; cooperativeness, C; self-transcendence, ST)[215,216].

c) *The 36-item Short-Form Health Survey (SF36, Italian version).* It is a self-reporting questionnaire aimed to assess patient's perception of his or her general health status and quality of life. It refers to the four weeks prior to the completion of the test, regardless of the type of pathology presented. It consists of 36 questions that cover the following 8 domains of health: vitality (energy and fatigue), physical functioning, physical role functioning, social functioning, general health, bodily pain, general mental health and emotional problems. The scores from each domain can be pooled into two components scores, namely Physical Component Summary (SF36-PCS) and Mental Component Summary (SF36-MCS). A third score concerns the change of self-perceived health status (Change Summary, SF36-CS)[217,218].

d) *The INTERdisciplinary MEDicine (INTERMED)*, in its Self-Assessment version (INTERMED-SA). It is a 27-items clinical multi-dimensional instrument, with good reliability and validity, measuring the bio-psycho-social complexity and healthcare needs of patients[219].

The assessment of the KP metabolites was performed according to the analytical method reported in Borsini and colleagues[220]. Briefly, the analyses of tryptophan (TRP), 3-hydrossikynurenine (3HK) and kynurenine (KYN), were performed using an Agilent HP 1200 liquid chromatograph (Agilent, Milan, Italy) consisting of a binary pump, an autosampler and a thermostated column compartment. Chromatographic separations were carried out using a Discovery HS-F5 column (150 -x4.6 mm, 5 µm, Supelco, Milan, Italy) using 0.1% formic acid in water and acetonitrile (ACN) as mobile phase. The HPLC analyses were carried out using a linear elution profile of 15 min from 5% to 90% of ACN. The column was washed with 90%

ACN for 3.5 min, then equilibrated for 5 min with 5% ACN. The flow rate was 0.5 mL/min. The injection volume was 40 mL. An Agilent 6410 triple quadrupole-mass spectrometer with an electrospray ion source operating in positive mode was used for detection.

Liquid chromatography, serological and psychometric tests were performed blind for anthropometric data and other clinical records.

MetS was diagnosed according to both the International Diabetes Federation Consensus Worldwide[221] and the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATPIII) criteria[75].

Statistical analysis

Statistical analysis was performed using STATA 13.1 software (Stata Corp., College Station, Texas) and Microsoft Excel. Mean, median, standard deviation (SD) and range were calculated for continuous variables. Frequencies and percentages were used for dichotomous variables.

To assess data distribution, Kolmogorov-Smirnov normality test (with the Lilliefors' correction) was used. For normally distributed variables, differences among groups were tested by means of Student's t-test. For not-normally distributed variables, Mann-Whitney Rank Sum Test was used. For categorical and dichotomous variables, Chi-square tests were performed to compare proportions in the number of observations. Finally, univariate and multivariate logistic regression analyses were performed as part of the inferential analysis. Sex (male/female) was selected as the response variable; all the above-mentioned clinical, biometric, serological, and psychometric variables were included in the analysis. The statistical significance was set at $p<0.05$.

3.3.3 RESULTS

126 patients were enrolled in the study, 65 of whom (51.59%) were male, with a mean age of 59.88±11.70 years, and 61 (48.41%) were female, whose mean age was 58.61±12.36 years. Demographic, anthropometric and clinical characteristics of patients (both continuous and dichotomous variables) are summarized in Table 1.

Table 1. Description of the total sample.

Continuous Variables	**Mean**	**SD**	**Min**	**Max**	**N**
Anthropometric Variables					
Age (years)	59.26	12.00	26	82	126
Weight (kg)	75.50	11.58	44	127	126
Height (m)	1.68	0.09	1.5	1.91	126
BMI (kg/m^2)	26.53	5.04	17.18	44.82	126

Waist circumference (cm)	96.44	15.35	65	129	126
Clinical variables					
SBP (mmHg)	140.92	17.35	104	205	126
DBP (mmHg)	83.04	9.12	60	110	126
Glycemia (mg/dl)	95.95	20.07	64	197	118
Total Cholesterol (mg/dl)	203.28	35.22	118	302	113
HDL (mg/dl)	54.50	15.59	30	113.4	113
LDL (mg/dl)	125.79	28.14	61	205	113
TRG (mg/dl)	114.72	45.47	36	220	113
Psychometric variables					
HADS-A	6.92	5.18	0	23	99
HADS-D	4.79	3.72	0	19	99
TCI-NS	22.53	3.64	14	32	85
TCI-A	18.71	2.75	10	26	85
TCI-RD	11.52	2.77	6	18	85
TCI-P	5.25	1.14	3	8	85
TCI-SD	26.76	4.18	13	34	85
TCI-C	22.38	4.57	11	32	85
TCI-ST	20.85	3.46	9	28	85
SF36-CS	2.97	0.92	1	5	92
SF36-PCS	46.94	8.95	21.43	63.10	92
SF36-MCS	48.43	10.63	19.85	66.13	92
Serological variables					
hs-CRP (mg/L)	0.56	0.35	0.00	2.1	115
TRP (μM)	22.96	6.83	10.37	40.35	121
KYN (μM)	1.86	0.59	0.82	4.08	121
KYN/TRP	0.09	0.03	0.04	0.18	121
3HK (μM)	0.02	0.01	0.003	0.102	121
3HK/KYN	0.01	0.004	0.002	0.027	121
3HK/TRP	0.0009	0.00004	0.00009	0.0031	121
Dichotomous Variable		**Presence (n, %)**	**Absence (n, %)**		**N**
Male sex		65 (51.59%)	61(48.41%)		126
MetS (ATPIII)		54 (46.96%)	61 (53.04%)		115
MetS (IDF)		60 (52.17%)	55 (47.83%)		115

Hypertension	91 (72.80%)	34(27.20%)	125
Systolic Hypertension	84 (72.41%)	32 (27.59%)	116
Diastolic Hypertension	52 (44.83%)	64 (55.17%)	116
Hyperglycaemia	44 (37.29%)	74 (62.71%)	118
Waist circumference (ATPIII)	61 (48.41%)	65(51.59%)	126
Waist circumference (IDF)	87 (69.05%)	39 (30.95%)	126
Hypertriglyceridemia	34 (29.57 %)	81 (70.43%)	115
HDL Hypocolestherolemia	44 (38.26%)	71 (61.74%)	115
Total Hypercholesterolemia	26(49.12%)	58 (50.88%)	114
LDL Hypercholesterolemia	67 (58.77%)	47 (41.23%)	114
BMI > 25 kg/m^2	70 (55.56%)	56 (44.44%)	126
Anxiety Symptoms	41(41.41%)	58(58.59%)	99
Depressive Symptoms	22 (22.22%)	77(77.78%)	99
Anxious-depressive symptoms	13(13.13%)	86(86.87%)	99
CRAs	51(43.97%)	65(56.03%)	116
INTERMED>21	4 (4.49%)	85 (95.51%)	89
hs-CRP > 1 mg/L	37 (32.17%)	78 (67.83%)	115
Alcohol use	49 (38.89%)	77(61.11%)	126
Sedentary lifestyle	67(53.17%)	59(46.83%)	126

List of abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TRG, triglycerides; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale, Depression subscale; TCI, Temperament and Character Inventory; NS, Novelty Seeking; A, Avoidance; RD, Reward Dependence; P, Persistence; SD, Self-Directedness; C, Cooperativeness; ST, Self-Transcendence; SF36, 36-item Short-Form Health Survey; PCS, Physical Component Summary; MCS, Mental Component Summary; hs-CRP, high sensitivity C-Reactive Protein; TRP, tryptophan; KYN, kynurenine; 3HK, 3-hydrossikynurenine; MetS, Metabolic Syndrome; ATPIII, Adult Treatment Panel; IDF, International Diabetes Federation; CRAs, Colorectal Adenomas; INTERMED, The INTERdisciplinary MEDicine.

Table 2 displays the results at the Student's t-test for means' comparison of continuous normally distributed variables according to gender. Statistically significant differences are also displayed in figure 1.

Table 2. Comparison of continuous normally distributed variables according to gender (Student's t-test).

	Men (mean±SD)	**Women (mean±SD)**	**t**	**p**
Age (years)	60.08±11.72	58.02±12.36	-.957	0.34
Weight (kg)	82.13±16.03	68.23±16.36	-4.807	**0.00**
BMI (kg/m^2)	27.30±4.53	25.66±5.46	-1.827	0.07
Waist Circumference (cm)	101.08±13.05	91.77±15.84	-3.580	**0.00**
SBP (mmHg)	141.77±16.86	140.02±17.84	-.566	0.57
HDL (mg/dl)	50.19±13.14	59.38±16.80	3.209	**0.00**
HADS-A	4.72±3.84	7.21±4.74	2.674	**0.01**
TCI-NS	21.80±3.58	22.86±3.47	1.280	0.20
TCI-A	18.40±2.69	18.92±3.06	.765	0.45
TCI-RD	10.40±2.42	12.14±2.47	3.011	**0.00**
TCI-C	22.63±4.51	22.32±4.71	-.28	0.78
SF36-PCS	49.16±7.54	46.67±9.65	-1.28	0.21
TRP (μM)	23.98±6.89	21.77±6.61	-1.8	0.07
KYN (μM)	1.86±0.49	1.85±0.69	-0.004	1
KYN/TRP	0.08±0.03	0.09±0.04	1.15	0.25
3HK/TRP	0.0008±0.0003	0.001±0.0005	2.63	**0.01**
3HK/KYN	0.01±0.003	0.12±0.005	2.23	**0.03**

List of abbreviations: BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein cholesterol; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; TCI, Temperament and Character Inventory; NS, Novelty Seeking; A, Avoidance; RD, Reward Dependence; C, Cooperativeness; SF36, 36-item Short-Form Health Survey; PCS, Physical Component Summary; TRP, tryptophan; KYN, kynurenine; 3HK, 3-hydrossikynurenine.

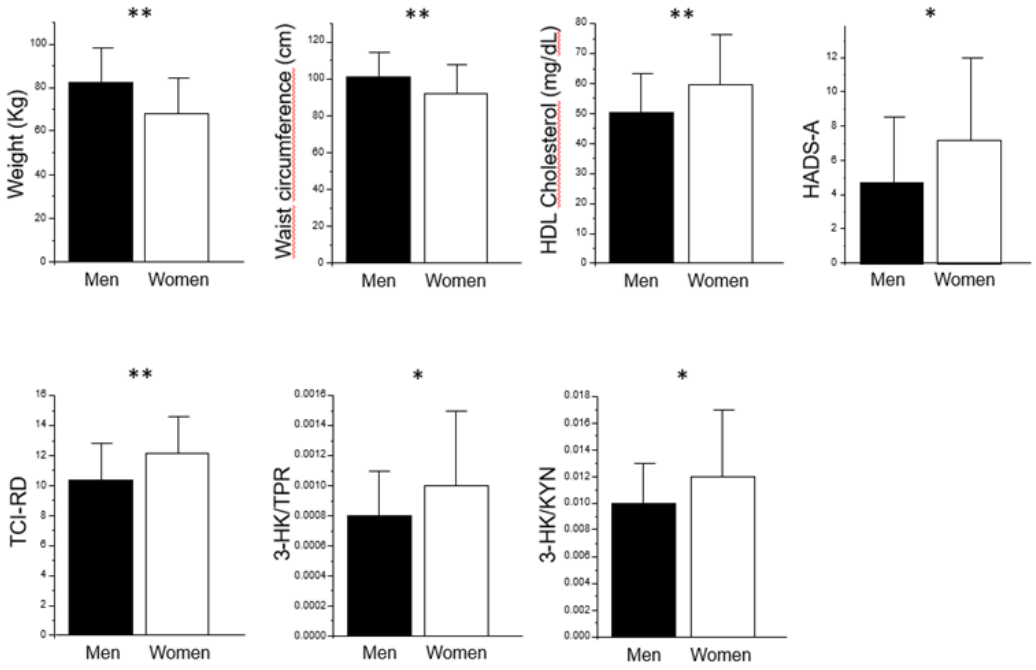


Figure 1. Statistically significant differences for normally distributed continuous variables according to gender. *=p<0.05; **=p≤0.01.

List of abbreviations: HDL, high-density lipoprotein cholesterol; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; TRP, tryptophan; KYN, kynurenine; 3HK, 3-hydrossikynurenine.

According to the U Mann-Whitney test for not normally distributed continuous variables, the distributions were different between males and females for SF36-MCS (men=52.11±7.91; women: 44.90±11.74; p=0.00) and diastolic blood pressure (men: 85.17±9.37; women: 80.85±9.90; p=0.04), as shown in table 3. Figure 2 also represents these differences.

Table 3. Comparison of continuous not normally-distributed variables according to gender (Mann-Whitney U test).

Continuous Variable	**Men (mean ±SD)**	**Women (mean ±SD)**	**p**
DBP (mmHg)	85.17±9.37	80.85±9.90	**0.04**
Glycemia (mg/dl)	95.43±19.93	96.48±20.37	.27
Total Cholesterol (mg/dl)	201.95±33.08	204.79±37.76	.79
LDL (mg/dl)	127.30±27.82	124.08±28.66	.21
TRG (mg/dl)	119.87±48.34	108.89±41.66	.23
HADS-D	4.47±3.66	5.1±3.79	.38
TCI-P	5.02±1.08	5.46±1.17	.16
TCI-SD	26.66±4.50	26.86±3.91	.74
TCI-ST	21±3.07	20.71±3.81	.87

SF36-MCS	52.11±7.91	44.90±11.74	**0.00**
hs-CRP (mg/L)	.61±.33	.51±.37	.09

List of abbreviations: DBP, diastolic blood pressure; LDL, low-density lipoprotein cholesterol; TRG, triglycerides; HADS-D, Hospital Anxiety and Depression Scale, Depression subscale; TCI, Temperament and Character Inventory; P, Persistence; SD, Self-Directedness; ST, Self-Transcendence; SF36, 36-item Short-Form Health Survey; MCS, Mental Component Summary; hs-CRP, high sensitivity C Reactive Protein.

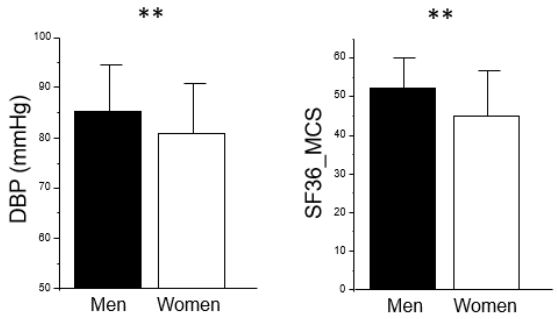


Figure 2. Statistically significant differences for not normally distributed continuous variables according to gender. **=p≤0.01.

List of abbreviations: DBP, diastolic blood pressure; SF36, 36-item Short-Form Health Survey; MCS, Mental Component Summary.

Table 4 shows the results of the gender-comparison for dichotomous variables as measured by means of Pearson's χ^2. Higher prevalence of CRAs (χ^2=9.32, p=0.00), diastolic hypertension (χ^2=8.88, p=0.003) and LDL hypercholesterolemia (χ^2=5.19, p=0.02) were detected in male than in female subjects. Anxiety (χ^2=5.30, p=0.02) and anxious-depressive (χ^2=4.07, p=0.04) symptoms were prevalent in women *vs*. men. Figure 3 also displays these statistically significant differences.

Table 4. Comparison of dichotomous variables according to gender (Pearson χ^2).

	Men (n, %)	**Women (n, %)**	**χ^2**	**p**
CRAs	34 (68%)	16 (32%)	9.32	**0.00**
HADS-A	9 (32.14%)	19 (67.86%)	5.30	**0.02**
HADS-D	8 (42.10%)	11 (57.90%)	0.61	0.44
HADS-AD	2 (20%)	8 (80%)	4.07	**0.04**
INTERMED	0 (0%)	2 (100%)	2.05	0.15
Alcohol use	31 (62%)	19 (38%)	3.08	0.08
Sedentary lifestyle	39 (58.21%)	28 (41.79%)	1.95	0.16
MetS-ATPIII	25 (48.08%)	27 (51.92%)	0.98	0.32

MetS-IDF	27 (48.21%)	29 (51.79%)	1.06	0.30
hs-CRP	22 (59.46%)	15 (40.54%)	1.16	0.28
Hypertension	46 (53.49%)	40 (46.51%)	0.24	0.62
Hyperglycaemia	21 (46.67%)	24 (53.33%)	0.74	0.39
Waist-circumference (ATPIII)	27 (44.26%)	34 (55.74%)	3.13	0.07
Waist-circumference (IDF)	43 (48.32%)	46 (51.68%)	2.01	0.16
HDL hypocholesterolemia	16 (32%)	21 (68%)	2.15	0.14
Iper-trygliceridaemia	21 (63.64%)	12 (36.36%)	2.08	0.15
Total Hypercholesterolemia	31 (56.36%)	24 (43.64%)	0.46	0.50
LDL Hypercholesterolemia	41 (62.12%)	25 (37.88%)	5.19	**0.02**
BMI>25	40 (57.97%)	29 (42.03%)	1.92	0.17
DBP	34 (68%)	16 (32%)	8.88	**0.003**
SBP	45 (52.33%)	41 (47.67%)	0.003	0.96

List of abbreviations: CRAs, Colorectal Adenomas; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale, Depression subscale; HADS-AD, Hospital Anxiety and Depression Scale, Anxiety and Depression total score; INTERMED, The INTERdisciplinary MEDicine; MetS, Metabolic Syndrome; ATPIII, Adult Treatment Panel; IDF, International Diabetes Federation; hs-CRP, high sensitivity C-Reactive Protein; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

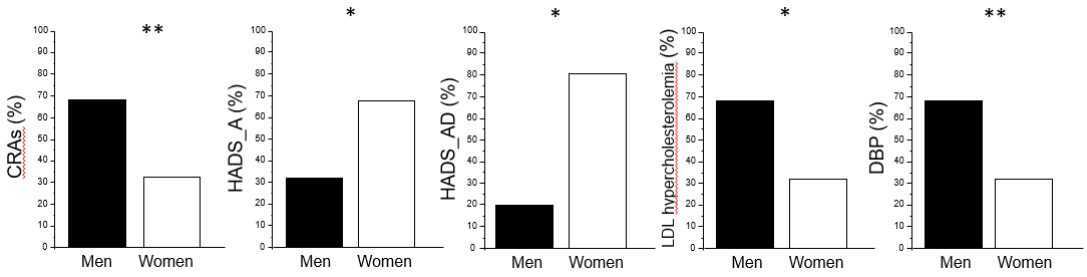


Figure 3. Statistically significant differences for dichotomous variables according to gender. *=p<0.05, **=p≤0.01.

List of abbreviations: CRAs, Colorectal Adenomas; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; HADS-AD, Hospital Anxiety and Depression Scale, Anxiety and Depression total score; LDL, low-density lipoprotein cholesterol; DBP, diastolic blood pressure.

A statistically significant difference between men and women was detected for the prevalence of visceral obesity (waist circumference above the ATPIII cut-off) in the subgroup of patients affected by anxiety (female: 93.85%, male: 6.3%; χ^2=11.48, p<0.01) and among patients with anxious-depressive symptoms (women: 88.9%, men: 11.1%, χ^2=4.44, p=0.04).

At the Mann Whitney U test for not normally distributed continuous variables, among patients with depression, TCI-Reward Dependence (p=0.03) and SF36-Phisical Component Summary (p<0.01) were significantly different between men and women.

Table 5 displays the statistically significant results of the univariate and multivariate logistic regression analyses, referring to male gender as response variable.

Table 5. Statistically significant results of the univariate and multivariate logistic regression analysis.

UNIVARIATE LOGISTIC REGRESSION ANALYSIS			
Male gender	**OR**	**p**	**95%CI**
CRAs	3.27	**<0.01**	1.51-7.08
HADS-A	0.87	**0.01**	0.79-0.97
HADS-AD	0.92	**0.02**	0.85-0.99
TCI-RD	0.74	**<0.01**	0.60-0.92
SF36-MCS	1.09	**<0.01**	1-03-1.15
Weight	1.06	**0.00**	1.03-1.15
Waist Circumference	1.05	**<0.01**	1.02-1.07
Blood HDL Cholesterol	0.958	**<0.01**	0.93-0.99
Diastolic Hypertension	3.19	**<0.01**	1.47-6.91
MULTIVARIATE LOGISTIC REGRESSION ANALYSIS			
TCI-RD	0.67	**0.04**	0.46-0.97
HDL Cholesterolemia	0.94	**0.02**	0.89-0.99
Diastolic Hypertension	10.14	**<0.01**	2.26-45.48

List of abbreviations: CRAs, Colorectal Adenomas; HADS-A, Hospital Anxiety and Depression Scale, Anxiety subscale; HADS-AD, Hospital Anxiety and Depression Scale, Anxiety and Depression total score; TCI, Temperament and Character Inventory; RD, Reward Dependence; SF36, 36-item Short-Form Health Survey; MCS, Mental Component Summary; HDL, high-density lipoprotein cholesterol.

3.3.4 DISCUSSION

This study aimed to investigate gender differences in anxious and/or depressive symptoms, MetS and its components, and CRAs in a sample of outpatients undergoing colonoscopy described adopting an integrated PNEI perspective.

In line with our previous findings that included a smaller sample[201,128], the prevalence of MetS among our sample of outpatients undergoing colonoscopy (49.96% according to ATPIII criteria, and 52.17% according to IDF definition) was higher than the one resulting from epidemiological data measured in general population[92]. This finding, though, may represent a selection bias, related to a possibly greater prevalence of MetS among people addressed to

colonoscopic screening, given that the association of CRAs and MetS is widely established in the literature[199].

The mean BMI of the total sample was 26.53 kg/m^2, corresponding to an overweight condition (BMI>25 kg/m^2) affecting more than half (55.65%) of the enrolled patients, a result that is slightly higher than the 43% reported by the PASSI (*Progressi delle Aziende Sanitarie per la Salute in Italia*, Progress of Health Authorities in Italy) data for the three-year period 2007-2009 among adults in Emilia-Romagna[222]. This difference, again, could be due to the fact that patients undergoing colonoscopy could have a higher BMI, another well-known risk factor for CRAs[223].

CRAs were detected in more than 40% of the sample (43.97%), a result that is higher than figures from the literature[224]. This was expected, considering that: 1) the sample was made of people undergoing screening colonoscopy for the detection of colorectal lesions; and 2) almost half of the sample had MetS, as said well-recognized significant risk factor for CRC and CRAs[200]. Moreover, the high prevalence of unhealthy lifestyles in our sample, as showed by the percentages of physical inactivity (53.17%) and alcohol consumption (38.89%) – all notorious cancer risk factors[224] – could further explain and justify this finding. CRAs were significantly more prevalent in men (χ^2=9.32, p=0.00) and were statistically significantly associated to male sex at the univariate logistic regression analysis (OR=3.27; p<0.01), in line with epidemiological data showing that women have fewer CRAs and CRC than men[206].

The gender differences detected in waist circumference (men: 101.08±13.05; women: 91.77±15.84; t=-3.58, p=0.00) and in HDL cholesterol mean concentrations (men: 50.19±13.14; women: 59.38±16.80; t=3.21; p=0.00) are consistent with the gender-different cut-points for the detection of abdominal obesity and HDL hypocholesterolaemia both according to IDF and ATPIII MetS definitions[221,75].

The condition of visceral adiposity (i.e. a waist circumference above the ATP-III criteria cut-off) was significantly prevalent in women with anxiety (χ^2=11.48, p=0.001) and with anxious-depressive symptoms (χ^2=4.44, p=0.035), in comparison with men affected by the same mental disorders. These data are similar to previous findings suggesting that increased waist circumference, an independent predictor of cardiometabolic disease[225,226] and the most convenient anthropometric correlate of visceral adipose tissue[226], was associated with an increased depression risk especially in women[227-230]. The association between increased waist circumference and anxiety in women is quite disputable: several studies found that anxiety symptoms are associated with variations in body weight and changes in BMI rather than with abdominal adiposity and increased waist circumference[230-232]; conversely, other studies detected a positive association between abdominal adiposity and anxiety, particularly in post-menopausal women[233,234].

The mean level of DBP and the prevalence of diastolic hypertension in our sample were higher in men than in women, consistently with previous evidence[235,236]. The association between male sex and diastolic hypertension at the multivariate logistic regression analysis is also consistent with existing data[236,237], suggesting the need for implementing gender-specific blood pressure guidelines. According to recent evidence, gender differences in sex steroids could mediate these associations, exacerbating cardiovascular disease in men.

To the best of our knowledge, few data are available on psychiatric symptoms and psychopathological characteristics of patients undergoing invasive procedures and colonoscopy, and the impact of emotional disorders on participation in screening colonoscopy is poorly characterized. According to the cross-sectional analysis by Calderwood and colleagues on a large cohort of adults without a history of CRC, depression seems not to be a risk factor for under-utilization of CRC screening[238]. Abgrall-Barbry et al. found that comorbid depressive mood may be associated with an increased likelihood of CRC in women undergoing colonoscopy for clinical reasons[239]. As far as our sample is concerned, despite the mean scores of anxious and/or depressive psychopathology were generally low, as expected in a non-clinical sample of outpatients, the prevalence of symptoms of anxiety and depression were higher than in the general population, in line with our previous research among smaller samples[201,128]. This result could be explained by several factors, including the use of different assessment methods (interview *vs.* self-report) and sampling strategy. Moreover, in our study, we administered the HADS, that is explicitly not intended to be a complete diagnostic instrument, but only a screening tool for symptoms of anxiety and/or depression, though very solidly accounted for[138,139,148].

Anxiety mean scores were significantly higher in females ($t=2.67$, $p=0.01$) than in male patients; moreover, both symptoms of anxiety ($\chi^2=5.30$, $p=0.02$) and symptoms of comorbid anxiety and depression ($\chi^2=4.07$, $p=0.04$) were significantly higher in women than in men. At the univariate logistic regression analysis, HADS-A (OR=0.87, p=0.01) and HADS-AD (OR=0.92, p=0.02) were inversely associated to male sex. All these results are in line with the literature, where epidemiological sex differences in anxiety and depressive disorders are clearly documented, independent of race or ethnicity, both in clinical samples[240] and in general population[182].

As far as personality is concerned, significant sex differences were observed in the TCI-RD subscale, with higher scores detected in women than in men, in accordance with previous records[241-245]. At the multivariate logistic regression analysis, TCI-RD was inversely associated with male sex. TCI-RD investigates sentimentalism, empathy, and intensity in reward-dependent responses (such as social approval and social support); such traits would fit with the macrosocial characteristics of Western culture as regards the distinction of sexual

roles and could also explain the higher predisposition of women towards stress-related diseases[182].

Finally, women reported lower SF36-MCS (p=0.00) and SF36-MCS (p=0.007) scores, suggesting that women generally self-perceived a worst mental and physical health status than men, consistently to previous studies investigating the impact of gender on health-related quality of life[246, 247].

With regards to KP metabolites, 3HK/KYN and 3HK/TRP ratios were significantly higher in women *vs.* men, suggesting a shift of the KP towards the synthesis of the neurotoxic metabolite 3HK. These data may mirror the findings of a previous study showing lower levels of the neuroprotective Kynurenic Acid (KYNA) and KYNA/3HK ratio in women compared to men[248].

Conversely to previous studies that demonstrated significant gender differences in the distribution of CRP[212], we did not find statistically significant gender differences in hs-CRP serum levels (p=0.09), and hs-CRP was not associated to sex at the regression analysis. This result may in fact be a consequence of the average low levels of hs-CRP, and to the limited sample size of the present study; further studies on larger samples could overcome this limitation.

A second limitation of this study concerns the psychometric assessment, performed using self-administered written questionnaires, rather than face-to-face clinical diagnostic interview, potentially leading to an over-diagnosis of anxiety and depression in our sample. Though it was specified that the HADS only detects psychiatric symptoms and not full-blown disorders, this tool is one of the most accredited and frequently used in research protocols similar to the present one, providing a reasonable combination of reliability and feasibility.

Thirdly, no control group was included, thus limiting the generalizability of our findings. Nevertheless, the sample was enrolled from outpatients performing screening procedures, thus minimizing selection bias from highly selected populations.

Finally, the cross-sectional design of the study does not allow to draw causal connections between the variables considered.

Notwithstanding these limitations, the present study represents a preliminary attempt to adopt a clinical multidimensional PNEI approach, increasingly accepted as a validated paradigm in the scientific community. Rather than focusing separately on each single disorders, the PNEI model can help clinicians and researchers in adopting a more holistic and multidisciplinary approach to their patients, taking into account the complex body-mind interconnections.

3.3.5 CONCLUSIONS

In conclusion, this study underlines sex differences detected in a sample of outpatients undergoing colonoscopy for screening reasons according to a multidisciplinary PNEI

perspective. Additional information on the role of gender differences in the association between anxious and depressive symptoms, MetS and CRAs could result in the implementation of gender-specific recommendations directed to the general population for screening strategies of these highly prevalent and frequently comorbid conditions.

3.4 STUDY IV

The role of chronic systemic low-grade inflammation and of the Kynurenine Pathway in the comorbidity between Metabolic Syndrome, anxiety and depression: preliminary findings from a cross-sectional study among outpatients.

These original data have not been published yet.

ABSTRACT

Background: Metabolic Syndrome (MetS) and anxious-depressive disorders are two of the most disabling disorders worldwide, often comorbid. The pathogenesis of this co-occurrence is complex and poorly researched. Dysregulation of inflammatory cytokines and of the kynurenine (KYN) pathway (KP) of tryptophan (TRP) metabolism were suggested as possible contributors to the pathogenesis of depression and anxiety, and seem to play a role also in MetS.

Aims: To explore the role of serum pro-inflammatory cytokines and KP metabolites in the comorbidity between anxiety and depressive symptoms and MetS among outpatients undergoing colonoscopy for screening reasons or abdominal symptoms at the Modena Policlinico General Hospital, Italy.

Methods: Serum blood concentration of KP metabolites, hs-CRP and IL-6 were measured. For the psychometric assessment, HADS and TCI were used. MetS was diagnosed both according to ATPIII and IDF criteria.

Results: 126 patients were enrolled. MetS (vs. non MetS) patients had different distribution of several KP metabolites. At the regression analysis, hs-CRP ($p<0.01$) and 3HK ($p<0.01$) were associated to depression, suggesting their potential role as non-invasive biomarkers.

Conclusions: These preliminary results suggest that pro-inflammation circulating cytokines and the KP's metabolites are involved in MetS, anxiety and depression. Further studies on larger samples could explore the potential role of these metabolites as possible early diagnostic markers of these conditions.

3.4.1 INTRODUCTION

Metabolic Syndrome (MetS) and anxious-depressive disorders are two of the most disabling disorders worldwide, often comorbid.

MetS refers to the co-occurrence of several well-known cardiovascular risk factors, including insulin resistance, obesity, atherogenic dyslipidaemia and hypertension[70]. Patients with MetS are two to four times more likely to suffer a stroke, and two times more likely to develop cardiovascular disease[249].

Major depression is a syndrome consisting of the presence of five of nine possible symptoms, according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), the diagnostic manual of the American Psychiatric Association[43]. With regard to specific symptoms, there must be the presence of a depressed mood *and*/*or* markedly diminished interest or pleasure in all or almost all daily activities. These symptoms must cause clinically significant distress or impairment in the person's function, and the symptoms must not be due to the direct physiologic effects of a substance or a general medical condition[43]. The global prevalence of depression is increasing worldwide, especially among outpatients, but only less than 65% of depressed patients are properly diagnosed and treated[250].

Anxiety disorders are the most common psychiatric disorders in outpatients[251]. However, only half of anxiety cases are recognized by primary care providers and only one third of the affected patients were offered drug treatment[252], causing significant distress and severe functional impairment[253].

The comorbidity between MetS and anxious-depressive symptoms has been seldom investigated in literature, and with confounding results. Some studies found a significant bi-directional association between depression and MetS[254] and between anxiety and MetS[255,256], while others failed to replicate these associations[88,89,134]. Therefore, these relationships remain not completely understood, likely due to the heterogeneity of MetS, depression and anxiety as complex, multifactorial disorders[96].

An accumulating body of evidence suggests that common biological mechanisms, such as a dysregulated immune system, hyperactivity of hypothalamic-pituitary-adrenal (HPA) axis, chronic inflammation and the activation of the Kynurenine (KYN) pathway (KP) of tryptophan (TRP) degradation could underpin these comorbidities[96].

The KP has been hypothesized to be involved in the development and persistence of both MetS and anxious-depressive disorders[257]. In both conditions, inflammatory cytokines Interleukine-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-alpha) have been shown to activate indoleamine-2,3-dioxygenase (IDO), the main controller enzyme of the KP, causing the so-called "kynurenine shunt" and consequently reducing serotonin (5-HT) synthesis[209]. As shown in Figure 1, IDO activation converts TRP into an excitotoxic metabolite of the KP, 3-hydroxykynurenine (3HK), that is thought to increase with depression and anxiety. Further cleavage of 3HK by kynureninase yields 3-hydroxyanthranilic acid (3-HANA). 3-HANA can be oxidated to adenosine triphosphate (ATP) and picolinic acid (PICA); otherwise, 3-HANA can be converted in quinolinic acid (QUIN), another excitotoxic and neurodegenerative metabolite which can be degraded into nicotinamide adenine dinucleotide (NAD). Conversely, an alternative pathway is the conversion by kynurenine aminotransferases (KATs) of KYN to kynurenic acid (KYNA), a glutamate and 17-nicotinic acetylcholine receptor antagonist which has been hypothesized to be neuroprotective, balancing the neurodegenerative effect of

QUIN[258]. So, there is a balance between neurodegenerative and neuroprotective effects in the KP, expressed by the QUIN/KYNA ratio, strictly related to immune activation. Interestingly, QUIN/KYNA, as well as KYN/TRP ratio, could be both used as a proxy of the KP activation. Finally, 3HK can be even transformed by KATs into Xanthurenic Acid (XANA), a marker associated with IFN-alpha induced depression and insulin resistance[259].

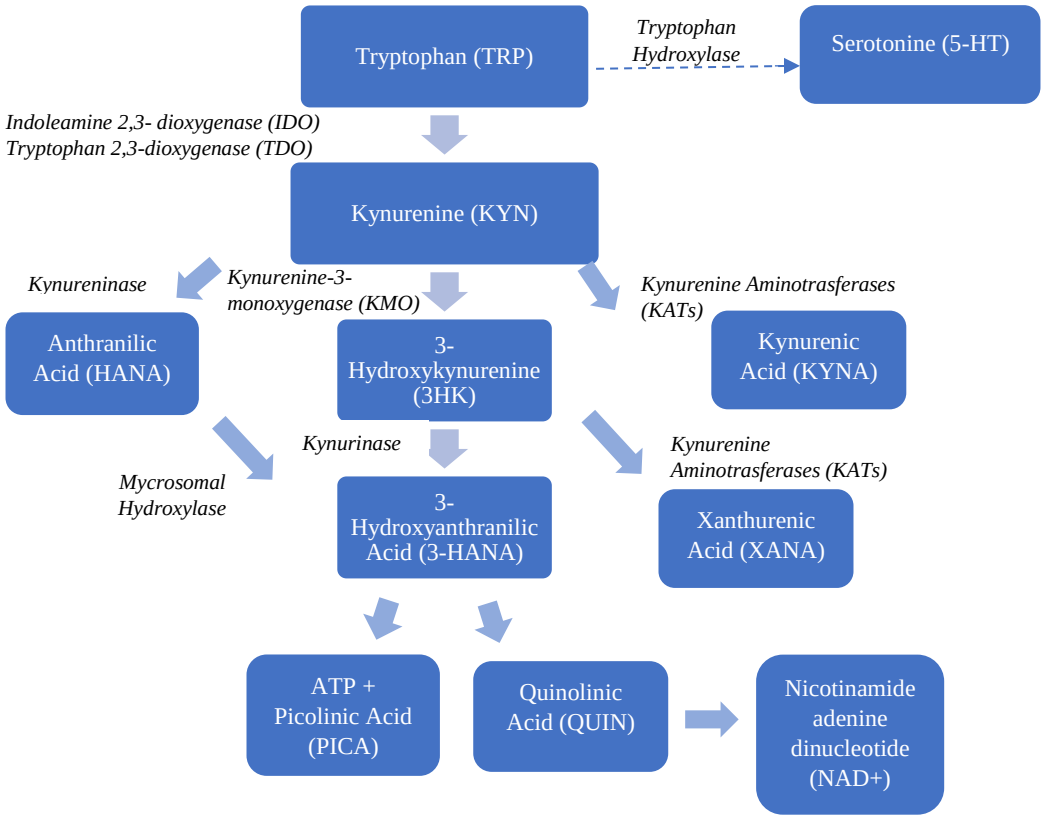


Figure 1. The Kynurenine pathway**.**

Abbreviations list: TRP, Tryptophan; IDO, Indoleamine 2,3-Dioxygenase; TDO, Tryptophan 2,3-Dioxygenase; KYN, Kynurenine; 3HK, 3-Hydroxykynurenine; KMO, Kynurenine-3-monooxygenase, KYNA, Kynurenic acid; KATs, kynurinine aminotransferases; HANA, Anthranlic Acid (HANA); 3-HANA, 3-hydroxyanthranillic acid; XANA, Xanthurenic acid; PICA, picolinic acid; QUIN, quinolinic acid; NAD+, Nicotinamide adenine dinucleotide.

Emerging evidence indicates that increased serological concentrations of KP's catabolites are associated with cardiovascular diseases, atherosclerosis and MetS components, including hypertension, diabetes and obesity[260].

For example, several studies found that KYN levels[260-263] and KYN/TRP[264,265] were higher in MetS patients versus healthy controls. As far as MetS components are concerned, obesity and overweight were related to increased KYN levels[266-269] and to higher KYN/TRP[266,270]. Circulating levels of KYNA and QUIN were positively related to higher BMI[269]. Higher

KYN/TRP[271,272], reduced TRP[259] and increased levels of KYN, KYNA, 3HK and XANA were detected in diabetic patients in comparison with healthy controls[273,259]. XANA was also associated with systolic (SBP) and diastolic (DBP) blood pressure in patients affected by hypertension *vs.* healthy controls[274]. With regards to dysplipidaemia, another key feature of MetS, KYN/TRP correlated negatively to HDL Cholesterol and Triglycerides (TRG), and positively to LDL Cholesterol[275]. Enhanced IDO activity was associated with Intima-Media Thickness (IMT) and several cardiovascular risk factors (age, BMI; plasma lipids, CRP) in young and middle-aged individuals[276,260].

It has been hypothesized that all these metabolites might contribute to the development of MetS via their apoptotic, neurotoxic, and pro-oxidative effects, linking peripheral inflammation and central nervous system alterations[209].

The metabolites of the KP seem to play an important role also in depression and anxiety[277,278]. Higher QUIN/KYNA ratio were detected in depressed patients *vs.* healthy controls[278], suggesting an imbalance of KP metabolites towards neurodegenerative effects. Increased QUIN and 3HK circulating levels were found in patients with anxiety and depression compared to healthy controls[279,280]. Ogyu and colleagues, in a recent metanalysis[281], found decreased KYNA levels in antidepressant-free depressed patients in comparison with healthy controls. Higher KYN/TRP were associated with poorer cognitive performance in depressed patients[282]. Finally, alterations in sleep patterns in depressed patients were found to be associated with elevated QUIN/KYNA ratio[283].

Notwithstanding these literary findings, there are several discrepancies that should be taken into account. Some studies found no correlations or even a negative correlation between depression and activation of the KP. In a study among depressed patients compared with healthy controls, no differences in KYN and QUIN concentration were found[284]. Dahl and colleagues found no increase in KP metabolites in depressed patients in comparison with healthy controls[285]. A systematic review and metanalysis found that circulating KYN levels decreased in depressed patients in comparison with healthy controls[286]. Probably, such conflicting results could be due to various confounders, like age, sex, patients' metabolic status and study design.

As far as anxiety is concerned, accumulating evidence (especially from laboratory and pre-clinical studies) has indicated the modulatory effects of the KP on anxiety disorders. The increase of QUIN and the decrease of KYNA lead to an increase of endogenous anxiogenic metabolites, and it is likely to be a pathway for inducing or continuing anxiety[287]. KYN, 3HK and QUIN seem to act as excitants, convulsivants and anxiogens[288,289], while KYNA and XANA could have anti-excitatory neuroactivities, such as inhibitory, tranquillizing, anxiolytics and anticonvulsant effects[287]. The relationship between KYN and anxiety has been revealed in few studies with healthy volunteers and psychiatric patients. In an experimental

study, an anxiogenic dose of caffeine (25mg/kg) was administered to 15 healthy volunteers, and blood plasma KYN concentration markedly increased at the peak of anxiety and returned to normal after anxiety had abated[290]. In a study with 30 psychiatric patients with affective state, patients with endogenous anxiety showed an increased plasma KYN concentration, that returned to normal after pharmacological anxiolytic treatment[291].

In summary, the literature on the association between anxiety, depression and MetS in clinical samples remains unclear. Alterations in inflammatory cytokines and in KP are likely to play a role in the comorbidity between these conditions, but an exact etiological role seem complex and requires further research.

Therefore, aims of the present study were to measure circulating pro-inflammatory cytokines and metabolites of the KP and to investigate their possible role in MetS, anxiety and depression in a sample of outpatients undergoing colonoscopy for non-specific abdominal symptoms.

3.4.2 MATERIAL AND METHODS

Study design

Cross-sectional study.

Ethics

All patients enrolled in the present study were asked to provide written informed consent. The study protocol was approved by the local Ethics Committee (Comitato Etico Provinciale di Modena, prot. No. 4396/C.E.). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and with Good Clinical Practices.

Study population

126 consecutive outpatients, of both sexes, who underwent colonoscopy for non-specific abdominal symptoms at the Modena Policlinico General Hospital (Modena, Northern Italy) were enrolled in the study. Patient recruitment started on January 1st 2015 and ended in June 1st 2021. Preliminary and partial data on a smaller sample of 62 patients were already published in a previous paper[201].

Data collection

For each enrolled subject, the following data were collected:

- socio-demographical information: age (years), sex;
- anthropometric variables: weight (kg), height (m), waist circumference (cm), hip circumference (cm);
- clinical parameters: systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg);
- a complete medical and pharmacological history, with detailed information about on-going drug therapies;

- lifestyle information, based on patients' account: active tobacco smoking, alcohol intake (2-3 drinks per week), sedentary habits (less than 30-min walk exercise at least 3 times per week).

BMI was calculated as body weight in kg/height in m^2. Waist to hip ratio (WHR), calculated as waist measurement divided by hip measurement, was used as a measure of abdominal adiposity.

For MetS assessment, both the National Cholesterol Education Program-Adult Treatment Panel III (ATPIII) and the International Diabetes Federation (IDF) criteria were used.

Lipid profile and high sensitivity C-Reactive Protein (hs-CRP)

Before the colonoscopy, a fasting blood draw was obtained in order to assess a complete standard lipid profile, blood glucose and hs-CRP dosage. Lipid profile included total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (TRG). Tests were performed on automated instrumentation Roche / Hitachi (Cobas 6000 Modular Analytics, F. Hoffmann-La Roche AG, Basel, Switzerland). Hs-CRP was measured by immunoturbidimetric technique with monoclonal antibodies bound to latex particles (dual-radius technology enhanced latex).

Enzyme Linked ImmunoSorbent Assays (ELISA)

A highly sensitive quantitative sandwich enzyme immunoassay technique, Quantikine® HS ELISA KIT (R& D systems, Minneapolis, USA), was used to measure circulating serum levels of IL-6 in accordance with the manufacture's instruction starting from 200 μL of diluted sera or standard solutions. After adding Stop Solution to each well and the optical density was measured by using a Thermo Scientific Multiskan® FC microplate reader, at 490 and 690 nm. To measure circulating levels of LPS, the human lipopolysaccharides/LPS ELISA kit (CUSABIO) was used, according to the product instructions, starting from 100 μL of diluted sera or standard solutions. TNF-and IFN-levels were also measured in the serum of patients by using comedically available highly sensitive Picokine ELISA KITs, but the cytokine levels were under the limit of detection of these KIT.

Liquid chromatography

The liquid chromatography analyses were performed according to the methods described by Borsini and colleagues[220].

Briefly, the analyses of serotonine (5-HT), tryptophan (TRP), 3-hydrossikynurenine (3HK), kynurenine (KYN), kynurenic acid (KYNA), 3-hydrossianthranilic acid (3-HANA), anthranilic acid (ANA) picolinic acid (PICA), xanthurenic acid (XANA) and quinolinic acid (QUIN) were performed using an Agilent HP 1200 liquid chromatograph (Agilent, Milan, Italy) consisting of a binary pump, an autosampler and a thermostated column compartment. Chromatographic separations were carried out using a Discovery HS-F5 column (150 - 4.6 mm, 5 μm, Supelco, Milan, Italy) using 0.1% formic acid in water and acetonitrile (ACN) as

mobile phase. The HPLC analyses were carried out using a linear elution profile of 15 min from 5% to 90% of ACN. The column was washed with 90% ACN for 3.5 min, then equilibrated for 5 min with 5% ACN. The flow rate was 0.5 mL/min. The injection volume was 40 mL. An Agilent 6410 triple quadrupole-mass spectrometer with an electrospray ion source operating in positive mode was used for detection. The optimized source parameters for MS analysis were: drying gas temperature 350 °C and gas flow 12 L/min, nebulizer gas flow pressure 35 psi and capillary voltage 4500 V. The SRM pairs were 177->160, 205–>188, 209–>192, 190->144, 206->160, 138->120, 225->208, 154->136, 168->78 AND 124->106 for 5-HT, TRP, KYN, KYNA, XANA, ANA, 3HK, 3-HANA, QUIN and PICA respectively. The calibration curves were constructed using seven calibration standards and were linear over the concentration range of 0.001-1 μM for 5-HT, 3HK, 3-HANA, KYNA, PICA, XANA and ANA, 0.005-5 μM for KYN and QUIN and 0.1-100 μM for TRP, with a correlation coefficient (r2) of about 0.99 for all analytes. As internal standard (IS) d5-TRP, d3-QUIN, d5-KYNA, d4-KYN purchased for Buchem BV (Netherlands) were used. All other drugs and reagents were purchased from Sigma–Aldrich (St Louis, MO, USA).

Immunohistochemistry and scoring for Myeloperoxidase and Autophagy expression

During colonoscopy, three microbiopsies of the ascending, descending, and sigmoid colon were performed and analyzed for evaluating the expression of a marker of autophagy (AUT) and MPO) on right colon (AUT1), left colon (AUT2) and the sigmoid-rectum (AUT3), respectively. AUT was measured as immunofluorescence intensity (IF), calculated as the average for each selected area, considering that an intensity value ranging from 0 (black) to 255 (white) was assigned to each pixel. The fluorescence intensity at the selected areas, linearly correlated with the number of pixels, was quantitatively analysed using the standard imaging analysis software of an NIS-Elements System. To each sample was assigned a code number and the score, referred to as Immuno-Fluorescence Intensity Score (IFIS), was determined.

The expression of myeloperoxidase (MPO), a marker of subclinical mucosal inflammation, was calculated as the mean number of positive cells per optical field (cells/OF). Before immunohistochemistry, a routine histology of tissue samples was carried out after hematoxylin and eosin staining of the sections. To assess the number of MPO-positive cells expressed in the colorectal mucosa, a quantitative score was used, counting the number of stained cells by immunohistochemistry under a light microscope. Two slides for each sample (about three samples for each patient) were scored by the analysis of at least 25 microscopic fields (magnification ×100). Cells located in capillary vessels were excluded by the observers. Slides were scored by two independent observers. Then, the mean number of MPO-positive cells (positive cells/OF) for each patient were calculated for each large bowel segment: right colon

(cecum to proximal half of the transverse colon, MPO1), left colon (distal half of the transverse colon and descending colon, MPO2), and the sigmoid-rectum (MPO3).

Psychometric assessment

After colonoscopy, each patient was asked to fulfil several self-administered tests for the psychometric assessment.

The following tests have been used in their validated Italian-language versions:

1) Hospital Anxiety and Depression Scale, HADS. It is a 14-item self-report instrument to screen for the presence and severity of symptoms of depression and anxiety over the past week. 7 items screen for anxiety and 7 for depression, with a cut-off for the symptoms anxiety and depression set at score ≥ 8 for both items[138,139].

2) *Temperament and Character Inventory, TCI*. It is a 240-item self-report questionnaire, investigating personality features of patients that may play a role in explaining bio-psycho-social complexity. TCI assess seven dimensions of personality: four so-called temperaments, namely novelty-seeking (TCI-NS), harm avoidance (TCI-HA), reward dependence (TCI-RD), persistence (TCI-P), and three so-called characters, namely self-directedness (TCI-SD), cooperativeness (TCI-C), and self-transcendence (TCI-ST). Noteworthy, TCI it is not an instrument aimed at the diagnosis of personality disorders, yet is useful in order to outline traits and dimensions of the personality[215,216].

3) Medical Outcome Study Short-Form 36, SF36. It is a questionnaire for self-administration measuring health-related quality of life, referring to the four weeks prior to the completion of the test. The questionnaire investigates eight domains: physical functioning, general health, physical role, bodily pain, mental health, social functioning, vitality/fatigue, and emotional role. The data from these categories can be divided into two main categories: a score for Physical Component Summary (SF36-PCS) and a score for Mental Component Summary (SF36-MCS). A third category, concerning the change of health status (Change Summary, SF36-CS), can also be derived[218].

4) INTERMED, INTERdisciplinary MEDicine, in its Self-Assessment version (INTERMED-SA). It is a multi-dimensional tool intending to identify the bio-psycho-social complexity and health care complex needs of patients. The tool is organized in two dimensions: the first dimension has four areas that reflect the biological systems, psychological, social welfare and health of the individual; the second dimension organizes time in the three periods of history, current status and prognosis. INTERMED scores of 21 or higher identify a highly complex patient[219].

Serological, psychometric and immunohistochemistry tests were performed in blind for anthropometric data and other clinical records.

Statistical analysis

Statistical analysis was performed using STATA 13.1 and Microsoft Excel software.

Continuous variables were expressed as mean ± standard deviation (SD). To assess data distribution, Kolmogorov-Smirnov normality test (with the Lilliefors' correction) was used. For normally distributed variables, differences among groups have been tested through Student't-test; for not-normally distributed variables, Mann-Whitney Rank Sum Test was used. For dichotomous variables, Chi-square test were performed to compare proportions in the number of observations. Spearman Rank Order Correlation was used to measure the strength of association between pairs of continuous variables.

In order to define the relative weight and the best predictors among the variables of the presence of symptoms of anxiety and depression, respectively, two backword multiple linear regression analyses were performed. For the inferential analysis, the statistically significance was set at $p < 0.05$.

3.4.3 RESULTS

126 patients were enrolled in the study, whose 65 (51.59%) were male, with mean age of 59.88±11.70 years, and 61 (48.41%) were female, whose mean age was 58.61±12.36 years.

54 (46.96%) had MetS according to ATPIII definition, and 60 (52.17%) according to IDF definition. 70 patients (55.56%) were overweight.

At least one CRA was detected in 51 patients (43.97% of the total sample) at the colonoscopy.

Forty-four patients (41.41%) had symptoms of anxiety, 22 (22.22%) reported symptoms of depression and 13 (13.13%) had concomitant anxious-depressive symptoms. 49 (38.89%) of the patients drunk excessively alcohol and 67 (53.17%) had a sedentary lifestyle.

Demographic, anthropometric, and clinical characteristics of the patients, as far as continuous variables are concerned, are displayed in table 1.

Table 1. Description of the sample (continuous variables).

Continuous Variable	**Mean**	**SD**	**Min**	**Max**	**N**
Age (years)	59.26	12.00	26	82	126
Weight (kg)	75.50	11.58	44	127	126
Height (m)	1.68	0.09	1.5	1.91	126
BMI (kg/m^2)	26.53	5.04	17.18	44.82	126
Waist circumference (cm)	96.44	15.35	65	129	126
SBP (mmHg)	140.92	17.35	104	205	126
DBP (mmHg)	8.08	9.84	60	110	126
Glycemia (mg/dl)	95.95	20.07	64	197	118
Total Cholesterol (mg/dl)	203.28	35.22	118	302	113
HDL (mg/dl)	54.50	15.59	30	113.4	113

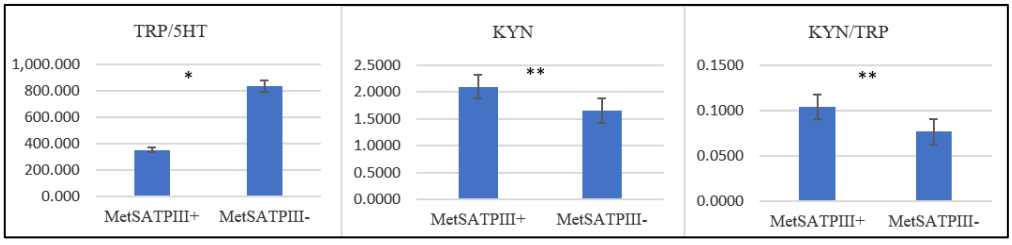
LDL (mg/dl)	125.79	28.14	61	205	113
TRG (mg/dl)	114.72	45.47	36	220	113
HADS-A	6.92	5.18	0	23	99
HADS-D	4.79	3.72	0	19	99
TCI-NS	22.53	3.64	14	32	85
TCI-A	18.71	2.75	10	26	85
TCI-RD	11.52	2.77	6	18	85
TCI-P	5.25	1.14	3	8	85
TCI-SD	26.76	4.18	13	34	85
TCI-C	22.38	4.57	11	32	85
TCI-ST	20.85	3.46	9	28	85
SF36-CS	2.97	0.92	1	5	92
SF36-PCS	46.94	8.95	21.43	63.10	92
SF36-MCS	48.43	10.63	19.85	66.13	92
hs-CRP (mg/L)	0.5617	0.3521	0.00	2.1	115
IL-6 (pg/ml)	4.2172	3.8736	0.7068	27.6497	95
LPS (pg/ml)	37.9124	26.1110	11.2630	96.4600	51
5-HT (μM)	0.1551	0.1273	0.0032	0.9431	118
TRP/5-HT	598.8427	1271.7680	16.5136	9067.0700	121
3HK(μM)	0.0202	0.0107	0.0033	0.1016	121
3HK/KYN	0.0111	0.0042	0.0018	0.0277	121
3HK/TRP	0.0009	0.0004	0.0018	0.0277	121
3HK/5-HT	0.4022	0.7034	0.018	4.5199	121
TRP (μM)	22.9605	6.8253	10.3705	40.3498	121
KYN (μM)	1.8547	0.5862	0.8129	4.0773	121
KYN/KYNA	76.4017	38.9311	17.4898	231.9287	121
KYN/TRP	0.0875	0.0345	0.0354	0.1839	121
KYNA (μM)	0.0326	0.0229	0.0062	0.1114	121
KYNA/QUIN	0.0806	0.0754	0.0097	0.5174	119
3-HANA (μM)	0.3593	1.7760	0.0027	18.1116	119
PICA/KYN	0.0225	0.1862	0.0012	0.1039	121
PICA (μM)	0.0386	0.2497	0.0010	0.1502	121
ANA (μM)	0.0491	0.2628	0.0047	0.1744	121
ANA/KYN	0.0276	0.0156	0.0044	0.1068	121
XANA (μM)	0.0128	0.0056	0.0021	0.0490	121

XANA/KYN	0.0073	0.0034	0.0015	0.0301	121
QUIN (μM)	0.5259	0.3652	0.0271	2.8124	119
KYN/QUIN	4.6601	3.8156	1.4375	38.9291	119
AUT1 (IF)	64.76	20.03	22	100	90
AUT2 (IF)	38.03	13.26	0	75	74
AUT3 (IF)	7	7.03	1	26	32
MPO1 (cells/optical field)	100.82	11.29	67	129	104
MPO2 (cells/optical field)	68.45	13.54	34	93	104
MPO3 (cells/optical field)	11.88	7.44	0	33	104

As shown in table 2 and in figure 2, the Student's t-test for means' comparison showed a statistically significant difference (not been due to simple chance) between patients with and without MetS according to ATPIII criteria for TRP-5HT (ATPIII-MetS+=352.31±666.49; ATPIII-MetS-= 834.29±1668.79; t=2.06, p=0.04), KYN mean (ATPIII-MetS+=2.10±0.63; ATPIII-MetS-=1.65±0.39; t=-4.42, p=0.00), KYN/TRP (ATPIII-MetS+=0.10±0.04; ATPIII-MetS-=0.08±0.03; t=-4.46, p=0.00) and KYNA mean (ATPIII-MetS+=0.04±0.02; ATPIII-MetS-=0.03±0.02; t=-2.63, p=0.01).

Table 2. Statistically significant results of the Student's t-test for means' comparison between patients with and without MetS according to ATPIII definition.

	ATPIII-MetS+ (mean ±SD)	**ATPIII-MetS- (mean ±SD)**	**t**	**p**
TRP/5HT	352.3142±666.4864	834.2857±1668.787	2.06	**0.04**
KYN (μM)	2.1013±0.6322	1.6547±0.3891	-4.42	**0.00**
KYN/TRP	0.1041±0.0351	0.0768±0.0290	-4.46	**0.00**
KYNA (μM)	0.0397±0.0229	0.0283±0.0228	-2.63	**0.01**



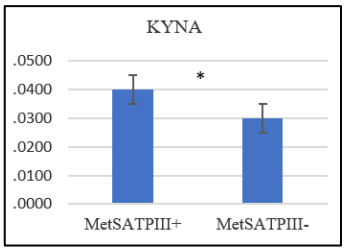


Figure 2. Statistically significant results of the means' comparison between patients with and without MetS according to ATPIII definition (Student's t-test).

According to the U Mann-Whitney test for not normally distributed continuous variables (see Table 3 and Figure 3), the distributions were different among patients with ATPIII-MetS *versus* patients without ATPIII-MetS for hs-CRP (p=0.001), 3HK/KYN (p<0.01), ANA (p<0.01) and QUIN (p<0.01).

Table 3. Statistically significant results of the U Mann-Whitney test for means' comparison between patients with and without MetS according to ATPIII definition.

	ATPIII-MetS+ (mean ±SD)	**ATPIII-MetS- (mean ±SD)**	**p**
hs-CRP (mg/L)	0.6788±0.3992	0.4721±0.2733	**0.001**
3HK/KYN	0.0099±0.0032	0.0118±0.0044	**<0.01**
ANA (µM)	0.0568±0.0266	0.0434±0.2391	**<0.01**
QUIN (µM)	0.6143±0.3326	0.4246±0.2367	**<0.01**

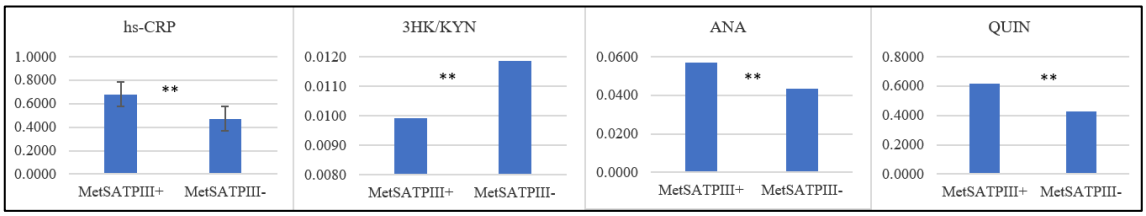


Figure 3. Statistically significant results of the means' comparison between patients with and without MetS according to ATPIII definition (U Mann-Whitney test).

As displayed in table 4 and in figure 4, the Student's t-test for means' comparison showed a statistically significant difference (not been due to simple chance) between patients with and without MetS according to IDF criteria for KYN mean (IDF-MetS+=2.08±0.62 IDF-MetS-=1.65±0.40; t=-4.38, p=0.00) and KYN/TRP (IDF-MetS+=0.10±0.04; IDF-MetS-=0.08±0.03; t=-4.11, p=0.00).

Table 4. Statistically significant results of the Student's t-test for means' comparison between patients with and without MetS according to IDF definition.

	IDF-MetS+ (mean ±SD)	IDF-MetS- (mean ±SD)	t	p
KYN (μM)	2.0772±0.6187	1.6469±0.3961	-4.38	**0.00**
KYN/TRP	0.1020±0.0350	0.0769±0.0294	-4.11	**0.00**

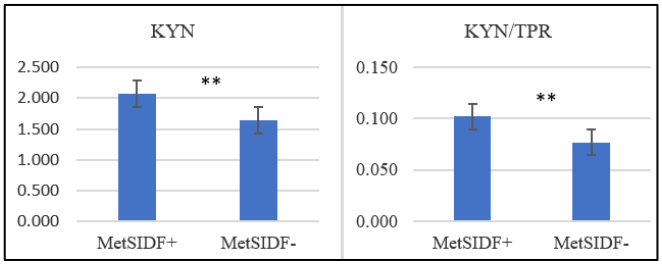


Figure 4. Statistically significant results of the means' comparison between patients with and without MetS according to IDF definition (U Mann-Whitney test).

According to the U Mann-Whitney test for not normally distributed continuous variables (table 5 and figure 5), the distributions were different among patients with MetS-IDF versus patients without MetS-IDF for hs-CRP (p<0.01), 3HK (p=0.02), ANA mean (p=0.01) and QUIN mean (p<0.01).

Table 5. Statistically significant results of the U Mann-Whitney test for means' comparison between patients with and without MetS according to IDF definition.

	MetS-IDF+ (mean ±SD)	MetS-IDF- (mean ±SD)	p
hs-CRP (mg/L)	0.6607±0.3916	0.4754±0.2805	**<0.01**
3HK (μM)	0.0102±0.0037	0.0117±0.0042	**0.02**
ANA (μM)	0.0552±0.2663	0.0440±0.0243	**0.01**
QUIN (μM)	0.6045±0.3239	0.4206±0.2422	**<0.01**

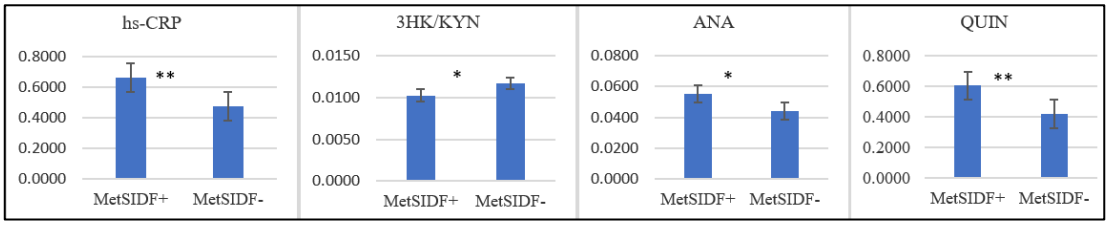


Figure 5. Statistically significant results of the means' comparison between patients with and without MetS according to IDF definition (U Mann-Whitney test).

The results of the correlation analysis (by means of Sperman's ρ) between kynurenines and inflammatory, psychometric and clinical-anthropometric variables are reported in Table 6, where the statistically significant correlations are highlighted in yellow.

Table 6. Correlation analysis

Pearson 's ρ		CRP	IL-6	LPS	5-HT	TRP/5-HT	3-OH-KYN	3-OH-KYN/KYN	3-OH-KYN/TRP	3-OH-KYN/5-HT	TRP	KYN	KYN/KYNA	KYN/TRP	KYNA	KYNA/QUIN	3-HANA	PICA	PICA/KYN	ANA	ANA/KYN	XANA	XANA/KYN	QUIN
HADS-A	ρ	,07	-,15	-,09	-,25	,23	-,01	,13	-,07	,24	,13	-,15	,33	-,21	-,36	-,06	-,10	-,04	-,04	-,21	-,12	-,18	-,06	-,31
	p	,48	,20	,56	,02	,02	,90	,20	,51	,02	,19	,13	,00	,04	,00	,55	,33	,69	,67	,04	,24	,07	,58	,00
HADS-D	ρ	,16	,27	,03	,06	-,05	,07	,02	,11	-,02	,03	,08	,11	,03	-,07	-,14	,04	-,12	-,11	-,04	-,09	-,04	-,07	,16
	p	,10	,02	,84	,54	,64	,48	,83	,26	,86	,78	,40	,29	,77	,49	,16	,67	,23	,29	,72	,37	,73	,49	,11
TCI-N	ρ	,06	-,03	,21	,07	,00	,15	-,06	,04	,00	,15	,16	,03	,02	,03	,14	,11	-,04	-,05	-,05	-,26	-,07	-,20	-,11
	p	,59	,83	,21	,54	,98	,17	,59	,71	,99	,17	,13	,79	,88	,78	,20	,30	,72	,63	,63	,02	,53	,07	,33
TCI-A	ρ	-,07	,04	,18	,07	-,07	,04	,13	,09	-,06	-,06	-,13	,10	-,07	-,12	-,16	,09	-,03	-,01	-,06	,00	-,13	-,04	,00
	p	,50	,74	,31	,53	,55	,70	,23	,43	,55	,59	,22	,38	,50	,27	,16	,43	,76	,95	,55	,97	,25	,71	,99
TCI-RD	ρ	-,24	-,01	,33	-,04	,02	,05	,08	,11	,06	-,01	,04	,04	,01	-,08	,06	-,19	-,21	-,15	,01	,04	-,16	-,06	-,16
	p	,03	,97	,05	,73	,87	,68	,44	,31	,56	,94	,74	,74	,90	,44	,58	,08	,05	,18	,91	,71	,15	,58	,15
TCI-P	ρ	-,16	-,11	-,03	-,02	-,01	-,12	-,17	,03	-,03	-,14	,07	-,09	,14	,07	,10	-,09	-,05	,01	,05	,00	-,07	-,04	,04
	p	,13	,38	,88	,84	,89	,28	,11	,80	,80	,20	,54	,43	,21	,52	,37	,42	,68	,90	,66	,97	,55	,72	,69
TCI-SD	ρ	,14	-,01	-,05	-,09	,10	,19	,00	,01	,16	,14	,15	,08	-,02	,01	-,05	,15	-,21	-,32	,06	-,01	-,03	-,04	,09
	p	,19	,96	,75	,44	,38	,08	,98	,95	,14	,21	,17	,48	,85	,95	,64	,18	,05	,00	,56	,93	,80	,71	,44
TCI-C	ρ	,01	,05	,11	-,07	,12	,08	,02	-,14	,08	,20	,00	,13	-,15	-,10	-,12	,19	-,09	-,16	-,02	-,03	-,04	,00	,00
	p	,94	,68	,52	,54	,27	,44	,84	,20	,45	,07	,98	,22	,16	,35	,30	,09	,40	,13	,84	,76	,69	,98	,97
TCI-ST	ρ	,20	,14	-,01	-,12	,16	-,09	-,08	-,25	,08	,20	-,05	,09	-,21	-,11	,01	,10	-,37	-,41	,03	,11	,03	,17	-,11
	p	,06	,25	,96	,28	,13	,40	,47	,02	,48	,07	,63	,41	,05	,32	,92	,39	,00	,00	,77	,31	,75	,12	,30
SF36-CS	ρ	,22	,34	,12	-,13	,13	,06	,14	,02	,16	,07	-,07	,16	-,12	-,17	-,07	,11	-,02	-,03	-,13	-,07	-,09	-,03	-,08
	p	,04	,00	,44	,23	,22	,59	,18	,84	,13	,48	,50	,12	,26	,11	,54	,31	,83	,81	,22	,52	,37	,79	,43
SF36-PCS	ρ	-,17	-,17	-,04	,12	-,09	-,15	-,19	-,17	-,17	,07	-,01	-,11	-,01	,12	,11	,20	-,03	-,05	-,12	-,14	,232*	,19	,00
	p	,11	,17	,78	,27	,38	,16	,07	,10	,11	,51	,96	,29	,92	,27	,31	,06	,76	,65	,25	,17	,03	,06	,99
SF36-MCS	ρ	,05	,02	,10	,13	-,21	-,08	-,09	,02	-,18	-,24	,02	-,31	,19	,27	,07	-,10	,14	,22	,19	,19	,06	,02	,21
	p	,66	,89	,53	,23	,05	,44	,42	,83	,09	,02	,88	,00	,07	,01	,54	,35	,17	,03	,06	,07	,59	,86	,05

Pearson 's ρ		CRP	IL-6	LPS	5-HT	TRP/5-HT	3-OH-KYN	3-OH-KYN/KYN	3-OH-KYN/TRP	3-OH-KYN/5-HT	TRP	KYN	KYN/KYNA	KYN/TRP	KYNA	KYNA/QUIN	3-HANA	PICA	PICA/KYN	ANA	ANA/KYN	XANA	XANA/KYN	QUIN
WEIGHT	ρ	,35	,28	,01	,06	-,02	,16	-,09	,01	,02	,09	,27	-,10	,12	,21	-,04	,22	-,06	-,11	,12	-,02	,15	-,08	,29
	p	,00	,01	,92	,49	,85	,08	,31	,90	,84	,35	,00	,29	,18	,02	,67	,02	,54	,25	,19	,85	,10	,41	,00
BMI	ρ	,34	,35	,05	,09	-,07	,23	-,11	,11	,00	,03	,36	-,09	,23	,25	-,06	,23	-,06	-,13	,17	-,03	,13	-,18	,37
	p	,00	,00	,72	,31	,47	,01	,25	,24	,98	,73	,00	,30	,01	,01	,51	,01	,51	,16	,06	,73	,15	,05	,00
WC	ρ	,42	,36	,07	,11	-,06	,25	-,10	,08	,02	,07	,38	-,07	,20	,23	-,07	,25	-,09	-,15	,17	-,03	,19	-,10	,36
	p	,00	,00	,63	,25	,48	,01	,30	,36	,80	,42	,00	,44	,03	,01	,45	,01	,31	,11	,06	,70	,04	,27	,00
HC	ρ	,39	,51	,05	,22	-,11	,27	-,15	,06	,02	,24	,359**	,10	,311*	,15	-,12	,02	-,40	-,43	,05	-,19	,06	-,32	,26
	p	,00	,00	,73	,09	,38	,03	,24	,65	,89	,06	,00	,45	,01	,24	,36	,88	,00	,00	,70	,14	,66	,01	,04
WHR	ρ	,43	,43	,00	,07	,04	,05	-,21	-,16	,03	,19	,13	,03	,04	,10	-,07	,24	,01	-,10	,11	-,06	,11	-,11	,17
	p	,00	,00	,99	,61	,79	,70	,11	,22	,85	,13	,33	,81	,76	,46	,61	,06	,97	,44	,40	,65	,38	,38	,20
SBP	ρ	,22	,11	-,13	,21	-,23	,06	-,20	,16	-,17	-,25	,29	-,27	,33	,36	-,02	,11	,08	-,02	,39	,25	,11	-,12	,38
	p	,02	,29	,35	,02	,01	,48	,03	,07	,06	,01	,00	,00	,00	,00	,81	,24	,40	,84	,00	,01	,21	,19	,00
DBP	ρ	,24	,18	-,09	-,03	,09	,10	-,07	-,04	,05	,11	,15	-,10	,00	,17	,02	,14	-,07	-,11	,17	,09	,13	,00	,13
	p	,01	,08	,53	,78	,33	,25	,44	,65	,58	,21	,09	,29	,96	,07	,82	,14	,45	,22	,07	,35	,15	,98	,17
Glycemia	ρ	,22	,28	,02	,07	-,05	,24	,10	,07	,03	,13	,189*	,16	,03	-,05	-,05	,15	-,13	-,17	-,06	-,16	,18	,04	,02
	p	,02	,01	,90	,47	,60	,01	,27	,43	,72	,17	,04	,10	,79	,58	,61	,12	,17	,07	,54	,09	,05	,70	,85
CHOL	ρ	,36	,32	,01	-,06	,12	,10	,05	-,17	,12	,26	,03	,20	-,17	-,13	-,13	,14	-,09	-,12	,01	-,04	,20	,12	,04
	p	,00	,00	,93	,53	,21	,28	,64	,07	,21	,01	,73	,03	,07	,18	,17	,15	,33	,20	,90	,71	,03	,19	,68
HDL	ρ	,05	,10	-,24	-,25	,287**	-,04	,08	-,22	,24	,203*	-,208*	,229*	-,28	-,26	-,11	-,14	-,12	-,11	-,04	,06	-,05	,06	-,17
	p	,57	,34	,08	,01	,00	,65	,40	,02	,01	,03	,03	,02	,00	,01	,27	,14	,22	,26	,64	,51	,60	,54	,07
LDL	ρ	,28	,31	,29	-,09	,13	,08	,12	-,15	,14	,25	-,04	,20	-,20	-,16	-,12	,14	-,07	-,06	-,11	-,13	,18	,18	-,02
	p	,00	,00	,04	,36	,17	,38	,20	,11	,15	,01	,68	,04	,03	,08	,23	,14	,44	,54	,25	,18	,05	,06	,80
TRG	ρ	0,19	,009	-,101	0,35	-,374**	,091	-0,29	,171	-0,33	-,212*	0,44	-0,22	0,43	0,38	,033	0,2	,114	,038	0,3	,058	,134	-0,2	0,39
	p	,04	,932	,479	,00	,000	,339	,00	,072	,00	,025	,00	,02	,00	,00	,735	,03	,232	,689	,00	,546	,159	,03	,00

As shown in table 7, two backward multiple linear regression analyses were performed to explore the association between symptoms of anxiety (HADS-A scores) and depression (HADS-D scores), respectively, and pro-inflammatory cytokines and KP metabolites. Table 7 reports the model with the best fit (adjusted R^2=0.295) for anxiety, and the best model for depression (adjusted R^2=0.229).

Table 7. Backward multiple linear regression analyses.

HADS-A scores	Unstandardized	Standard Error	Standardized	t	p	95%CI Lower Bound	95%CI Upper Bound
KYN/KYNA	0.12	0.03	1.04	4.59	<**0.001**	0.07	0.17
KYNA	82.94	39.06	0.42	2.12	**0.04**	4.80	161.07
PICA	396.38	140.30	1.54	2.83	<**0.01**	115.73	677.03
ANA/KYN	622.93	153.57	1.71	4.06	<**0.001**	315.75	930.12
HADS-D scores	Unstandardized	Standard Error	Standardized	t	p	95%CI Lower Bound	95%CI Upper Bound
hs-CRP	3.51	1.05	0.38	3.35	<**0.01**	1.42	5.61
3HK	552.62	171.86	1.36	3.22	<**0.01**	209.18	896.06
TRP	0.37	0.16	0.74	2.28	**0.03**	0.04	0.70
KYN/TRP	221.93	61.62	2.32	3.60	<**0.001**	98.80	345.06

3.4.4 DISCUSSION AND CONCLUSIONS

Aim of this study was to assess the role of KP metabolites and pro-inflammatory cytokines in MetS and symptoms of anxiety and depression among outpatients.

More than forty percent of the sample had symptoms of anxiety, about one fifth of the sample had symptoms of depression and 13.13% reported concomitant anxious-depressive symptoms. These results are substantially in line with our previous results in a smaller sample[201], suggesting that a psychometric assessment for common emotional disorders among outpatients undergoing screening procedures could be useful for the early detection and treatment of these highly prevalent conditions. The higher prevalence of these psychiatric symptoms in comparison with other data from general population[92] could be also due to the assessment methods (self-assessment questionnaires other than clinical interview) and to stressful socio-economical concomitant conditions (the economic crisis in Italy from 2009, and the COVID-19 pandemic) that had noticeable effects on mental health[292,293].

The prevalence of MetS varied from 46.96% (ATPIII criteria) to 52.17% (IDF criteria); 70 subjects (55.56%) had a BMI>25, corresponding to an overweight condition. These findings highlighted that MetS and overweight, two well-known risk factors for colorectal neoplasms[202,223], are highly prevalent among outpatients undergoing CRAs screening procedures. Therefore, targeted interventions on lifestyle and dietary habits in this population could be further implemented.

In comparison with patients without MetS, patients affected by MetS (both according to ATPIII and IDF definitions) had higher circulating levels of KYN, ANA and QUIN, suggesting that the chronic systemic inflammatory status detected in MetS patients might activate the KP, driving conversion of TRP to KYN and its metabolites, in accordance with previous literary evidence. Several papers, in fact, have shown an increase in KYN level in MetS patients[261-269]. The increase in ANA levels is in line with the hypothesis that ANA may contribute to disorders with an inflammatory component and may represent a novel marker for the assessment of inflammation and its progression[294]. The increase of QUIN has been already found in MetS patients[264], as well as in patients with higher BMI[269].

Compared to subjects without MetS, patients with MetS (both according to ATPIII and IDF definitions) had significantly higher hs-CRP circulating levels, a result in line with previous literary evidence[295] and that suggests hs-CRP as a possible novel marker for detecting individuals at risk for developing MetS.

At the correlation analysis, depressive symptoms (HADS-D) and SF36-CS correlated to IL-6. This finding is in accordance with previous studies indicating that increased peripheral or central IL-6 levels play an important role in stress reaction and depressive disorders[296, 303].

Symptoms of anxiety (HADS-A) and SF36-MCS correlated to several KP metabolites, suggesting an activation of the "TRP shunt" promoted by stress hormones and proinflammatory cytokines in these psychiatric conditions, a result in line with other literary evidence[287].

BMI, waist and hip circumference, WHR, glycemia, total cholesterol and LDL cholesterol correlated both to hs-CRP and IL-6; SBP, DBP and TRG correlated to hs-CRP alone. These results confirm the key role of inflammation in MetS and its components[297,298].

At the regression analysis, KYN/KYNA, KYNA, PICA and ANA/KYN were positively associated with to HADS-A, further confirming the activation of the KP in anxiety symptomatology[271].

Finally, at the regression analysis, 3HK, TRP and KYN/TRP were positively associated with HADS-D. The association between depressive symptoms and 3HK is in line with other papers[299, 303]. Elevated serum TRP may reflect the impairment of TRP conversion into 5-HT in agreement with suggested link between serotonin deficiency and depression. Up-regulation

of IDO and of the KP, whose KYN/TRP is a proxy, might be an additional risk factor for depression, as reported in literature[209, 303].

Briefly, the essential messages that can be derived from the present study are highlighted in table 8.

Table 8. Key messages.

KEY MESSAGES
• In comparison with patients without MetS, patients affected by MetS had higher circulating levels of hs-CRP, KYN, ANA and QUIN.
• HADS-A and SF36-MCS correlated to several KP metabolites, suggesting an activation of the "TRP shunt" promoted by stress hormones and proinflammatory cytokines.
• Several MetS single components (i.e. BMI, waist circumference, glycemia and LDL cholesterol) correlated both to hs-CRP and IL-6, suggesting the involvement of a pro-inflammatory systemic state in all these dysmetabolic conditions.
• At the regression analysis, hs-CRP was associated to HADS-D, suggesting the potential role of this cytokine as non-invasive biomarker.
• KYN/KYNA, KYNA, PICA and ANA/KYN were positively associated with to HADS-A and 3HK, TRP and KYN/TRP were positively associated with HADS-D, further confirming the activation of the KP in anxious-depressive symptomatology and opening new possible therapeutic and diagnostic perspectives.

Several limitations of the study must be acknowledged. First of all, the cross-sectional nature of the study does not allow to establish causal connections between the collected variables. Secondly, the limited sample size limits the generalizability of our results. Thirdly, this type of statistical analyses is susceptible to type I errors, which can lead to rejecting a null hypothesis when in fact it is true. Finally, the psychometric assessment was made by self-assessment questionnaires, and not with clinical interview, potentially influencing our results.

In conclusion, this study suggests that proinflammatory cytokines and several KP's metabolites are involved in MetS, anxiety and depression. Further studies on larger samples could explore the potential role of KP's metabolites as non-invasive markers for the detection of these conditions. The high prevalence of psychiatric symptoms detected in our sample could suggest the need to implement preventive and screening strategies among patients undergoing screening procedures.

4. OVERALL CONCLUSION

Given the global burden of anxiety and depression, MetS and CRAs on the general population, aims of these studies were to assess the prevalence and the association between these conditions, providing new insights into the potential mechanisms involved in their clinical co-occurrence.

Overall, these preliminary results pointed out an association between MetS, CRAs, symptoms of anxiety and depression that may be further studied in both epidemiological and PNEI perspectives on larger samples. The findings of our studies provide preliminary support that both CRAs and MetS might arise from complex and interconnected clinical and psychopathological substrates in humans. Nonetheless, our findings should be interpreted with caution considering the cross-sectional nature of the tested associations, not allowing any causal inference.

The high prevalence of anxious-depressive symptoms detected among out-patients highlight that such conditions deserve attention and adequate treatment. Gender-differences should be taken into account in order to implement effective prevention strategies directed to the general population.

Also MetS and overweight were highly prevalent conditions in our out-patients groups, suggesting the need to early detect and manage these dysmetabolic disorders in community-based settings. Future studies might assess the possible role of eating habits and hormonal mediators, such as leptin and ghrelin, in these comorbidities.

Moreover, our preliminary findings suggest a link between anxious-depressive symptoms, internal-dysmetabolic conditions and a chronic systemic proinflammatory state among outpatients, opening new perspectives for the prevention and treatment of these conditions. For example, pro-inflammatory cytokines such as CRP and IL-6 and several KP metabolites could be investigated as candidate non-invasive easy-measurable markers for the detection of MetS and symptoms of depression or anxiety. Furthermore, future studies are needed to clarify the role of the KP changes not only in serum, but also in various sample such as cerebrospinal fluid. Overall, a better understanding of the dynamics of the KP metabolites[300-303] may lead to the development of more sophisticated diagnostic and therapeutic strategies both in common emotional disorders and in dysmetabolic conditions.

Finally, our results suggest the clinical and scientific usefulness of implementing an integrated multidimensional PNEI model[304]. Rather than focusing separately on each single disorders, the PNEI model can help clinicians and researchers in adopting a more holistic and multidisciplinary approach to their patients, taking into account the complex body-mind interconnections.

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