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Circulating Blood B and T Lymphocytes and Severity of Acute Pancreatitis: A Systematic Review Protocol

Linfócitos B e T no Sangue Periférico e a Gravidade da Pancreatite Aguda: Protocolo de Revisão Sistemática

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ABSTRACT

Introduction: Acute pancreatitis is an acute inflammatory process of the pancreas with a high prevalence rate and varying degrees of severity that can be potentially life threatening. Much is still unknown about which mechanisms determine the course and severity of acute pancreatitis. The primary objective of this review is to identify the potential association between circulating B and T lymphocytes and the severity of acute pancreatitis. Subgroup analyses will be done according to the severity classification of the Revised Atlanta Classification System as well as according to the distinction between B lymphocytes and T lymphocytes and the severity of acute pancreatitis.

Methods: A systematic search will be performed in Medline, Web of Science, EMBASE, Cochrane Central Register of Controlled trials and ClinicalTrials.gov. Three authors will independently do the selection process as well as data extraction that will be recorded into a flow diagram following the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P). The pathophysiology of acute pancreatitis is still not fully understood and its evolution is sometimes unpredictable. In this context, through this systematic review, the research team intends to determine what has been described about the role of serum lymphocytes in determining the severity of acute pancreatitis, by identifying a potential indicator of the severity of this acute disease.

Keywords: B-Lymphocytes; Pancreatitis; Systematic Review; T-Lymphocytes

RESUMO

Introdução: A pancreatite aguda é uma doença inflamatória do pâncreas de elevada prevalência que pode evoluir com vários graus de gravidade e ser fatal. Muitos dos mecanismos que determinam a evolução e gravidade da pancreatite aguda ainda são desconhecidos. O objetivo principal desta revisão é identificar a potencial associação entre os níveis no sangue periférico dos linfócitos T e B e a gravidade da pancreatite aguda. Proceder-se-á também à análise por subgrupos de gravidade de acordo com os níveis de gravidade definidos pelo *Revised Atlanta Classification System* bem como a sua distinção de linfócitos T e linfócitos B.

Métodos: Será feita uma revisão sistemática na Medline, Web of Science, EMBASE, Cochrane Central Register of Controlled Trials e ClinicalTrails.gov. Três revisores farão de forma independente a seleção dos estudos bem como a extração de dados que serão registados em diagrama proposto pelo *Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols* (PRISMA-P). A pancreatite aguda é uma patologia com fisiopatologia ainda não totalmente esclarecida e evolução por vezes imprevisível. Neste contexto, através desta revisão sistemática a equipa de investigação pretende determinar o que há descrito sobre o papel dos linfócitos séricos na determinação da gravidade da pancreatite aguda, identificando um potencial indicador de gravidade desta doença aguda.

Palavras-chave: Linfócitos B; Linfócitos T; Pancreatite; Revisão Sistemática

INTRODUCTION

Acute pancreatitis is an inflammatory disease of the pancreas with an unpredictable course. This is one of the reasons why it is a leading cause of hospitalization from gastrointestinal diseases in Europe and the United States.[1,2] Acute pancreatitis can lead to significant morbidity as well as pancreatic insufficiency and long-term illness.[3]

We now know that injured acinar cells of the pancreas release chemokines leading to infiltration of immune cells, mainly neutrophils, with worsening tissue injury of the pancreas and systemic inflammation later on.[4] Neutrophils activate trypsinogen in acinar cells. These cells amplify the inflammatory cascade, generating many chemokines and cytokines including interleukins 1 and 6 (IL-1 and IL-6), and intercellular adhesion molecule 1 (ICAM-1) to promote pancreatic and extra-pancreatic multiorgan injury.[5] Disease severity depends on whether the inflammatory response resolves or expands.[6]

Predicting the severity of this disease as well as knowing its critical mechanisms is essential to better monitor and develop future treatments for patients who need the most, reducing morbidity and mortality of patients with acute pancreatitis. Several risk prediction scores, individual biomarkers and radiological scoring systems have been developed to predict outcomes. The Revised Atlanta Classification System, from 2012, defining the clinical diagnosis, computed tomography (CT) manifestations, and the disease course of acute pancreatitis is the most widely used in clinical practice.[7] This classification, evaluating additional local or systemic complications as well as the presence and duration of organ failure, divides acute pancreatitis into mild

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acute, moderately severe acute, and severe acute pancreatitis. However, this classification is only made when acute pancreatitis is already evolving and frequently when some of its complications are well established.

Other frequently used scores in clinical practice include calculation of body mass index and the APACHE II score and serum C-reactive protein. A few scoring systems including clinical and laboratory criteria have also been devised like the Bedside Index of Severity in Acute Pancreatitis (BISAP), and Ranson's criteria.[8] However, most of these scoring systems require 24 hours to predict severity, and several parameters are not easily available on admission. Therefore, early prediction of acute severity is still needed.[9]

As mentioned, acute pancreatitis is a disease characterized by local and systemic inflammation and the severity of this disease is associated with the systemic inflammatory response syndrome (SIRS).[10] The activation of the innate immune system has been well described in acute pancreatitis and it is known that the cascade of inflammation follows including the activation of the adaptative immune system.[5] Compensatory anti-inflammatory responses occur, shown by increases in regulatory T cells in lymphoid tissue, although this may turn out to be responsible for dysregulation instead of balance restoration and persistent inflammation or immunosuppression may prevail.[3]

Several studies have tried to relate the severity of acute pancreatitis and the different components of the innate immune system including c-reactive protein, cytokines but also lymphocytes.[11-14] These studies include the determination of blood B and T cells during the first week of hospitalization of patients with acute pancreatitis. It is known B and T cells play critical roles in the pathogenesis and severity of acute pancreatitis although their exact role has not yet been elucidated.[15,18] The neutrophil-to-lymphocyte ratio has also been indicated as a possible early marker of acute pancreatitis severity[16] mainly because this parameter has been shown to have significant correlation with systemic inflammation reaction in some autoimmune diseases.[17] However, and because broad-spectrum antibiotics, which are essential medicines in the treatment of severe acute pancreatitis, can affect neutrophil count by reducing inflammation, the neutrophil-to-lymphocyte ratio must be used with caution in determining the severity of acute pancreatitis in some clinical settings.[18]

So, by simply collecting a blood sample and evaluating the lymphocyte count, clinicians might have a useful tool for predicting the severity of acute pancreatitis.

The main objective of this work is to systematically review and summarize the current knowledge on the potential association between circulating B lymphocytes and T lymphocytes and relate it to the severity of acute pancreatitis.

Secondary objectives will include the exploration of the blood levels of B lymphocytes and T lymphocytes separately with the severity of acute pancreatitis using the Revised Atlanta Classification System.[7]

Therefore, this systematic review will focus on the role of these cells in helping to determine the severity of acute pancreatitis.

METHODS AND ANALYSIS

The study protocol has been pre-registered on PROSPERO (registration number CRD42023383303).

Eligibility criteria

This study will identify randomized controlled trials (RCT), cohort studies (prospective or retrospective) and case control studies that relate blood B lymphocytes and T lymphocytes to the severity of acute pancreatitis. We will exclude cross-sectional studies, case series and case reports.

We will include articles reported in the English language.

Studies on the adult human population (18 years and older).

No restriction regarding publication will be set. Therefore, studies will be included from inception to January 31, 2022 (Table 1).

Intervention exposure

Inclusion criteria: Human adults, hospitalized with the diagnosis of acute pancreatitis with blood collection to determine lymphocyte levels.

The diagnosis of acute pancreatitis will require the presence of two of the following three criteria: acute onset of persistent, severe epigastric pain often radiating to the back; elevation in serum lipase or amylase to three times or greater than the upper limit of normal; and characteristic findings of acute pancreatitis on imaging (contrast-enhanced computed tomography, magnetic resonance imaging, or transabdominal ultrasonography).

The severity of acute pancreatitis will be defined according to the classification of severity applied by the Revised Atlanta Classification System (mild acute pancreatitis, moderately severe acute pancreatitis and severe acute pancreatitis) and/or the following scoring systems: systemic inflammatory response syndrome (SIRS) score (≤ 2: mild pancreatitis[19]), the Acute Physiology and Chronic Health Examination (APACHE) II score (determined in the first 24 hours of admission score < 8 mild pancreatitis; score > 8 severe pancreatitis[20]), the Bedside Index of Severity in Acute Pancreatitis score (determined in the first 24 hours of admission BISAP: ≥ 3 severe pancreatitis[21]), Ranson's criteria (0 - 3 points: mild pancreatitis; ≥ 3 points: severe pancreatitis[22]), and the Computed Tomography severity index (score ≥ 6: severe pancreatitis[23]).

Table 1 – PICOST criteria for the inclusion of studies into the systematic review and meta-analysis

Criteria	Description
Participants	Adult human population (> 18 years old)
Exposure	Hospitalized patients with acute pancreatitis
Comparator	Not applicable
Primary outcome	Relate the change in peripheral blood lymphocytes and the severity of acute pancreatitis
Secondary outcome	Relate severity scores of acute pancreatitis with B lymphocytes and T lymphocytes separately
Study Design	Randomized controlled trials (RCT), cohort studies (prospective or retrospective), cross-sectional studies and case control studies
Timing	From inception to January 31, 2022

Exclusion criteria: < 18 years old, non-human, non-hospitalized.

Information sources and search strategy

We will conduct a comprehensive computerized literature search strategy to find the studies to be included in this systematic review. Published and unpublished studies will be searched in the following databases: PubMed/Medline, Web of Science, EMBASE and Cochrane Central Register of Controlled trials. The electronic database search will be supplemented by searching other electronic platforms such as ClinicalTrials.gov for ongoing or unpublished clinical trials.

If any relevant unpublished trial is found, the respective author will be contacted to obtain the necessary information and if the author does not respond or is not willing to share the required information, the trial will be excluded.

The search will include the following key words and all their variants, according to each database and its special requirements: “acute pancreatitis”, “severity”,” lymphocyte”, “B cell”, “T cell”, “immune cell’. Boolean operators like ‘OR’ or ‘AND’ will also be used (Table 2).

The reference list of the included articles will be searched to find eligible studies.

Study selection and data extraction

The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P)[24] will be followed in this systematic review.

Two reviewers will, independently and blind to each other, conduct the selection process. All records identified in the search stage will be screened by title/abstract and those not matching the criteria will be discarded. The remaining studies will be full-text reviewed and included or excluded according to the inclusion and exclusion criteria. If any disagreement arises between the reviewers, this will be solved by consensus or a third one if necessary.

The selection process as well as data extraction will be recorded into a flow diagram (Fig. 1).

Missing data will be requested from study authors. The excluded studies and the reasons for exclusion will be registered.

Data extraction will be done by two independent reviewers. Data extraction will include features of the study, patient characteristics, study methodology, times of measurement and outcomes. Discrepancies between the reviewers will be identified and solved by consensus or a third one if necessary.

Data management

Published studies will be imported to the Mendeley citation software where duplicates will be managed and discarded. Titles as well as abstracts of all records will be evaluated.

Risk of bias assessment

To minimize bias in the methodological quality of all studies included in this systematic review, the studies will

Table 2 – Search strategy for PubMed

Query	Search
1	“pancreatitis” (MeSH terms) OR “pancreatitis” OR “acute pancreatitis” (MeSH terms) OR “acute pancreatitis”
2	“severity” (MeSH terms) OR “severity” OR “severe” (MeSH terms) OR “severe”
3	“lymphocyte” (MeSH terms) OR “lymphocyte” OR “immune” (MeSH terms) OR “immune” OR “immune cell” (MeSH terms) OR “immune cell”
4	“T cell” (MeSH terms) OR “T cell” OR “CD4” (MeSH terms) OR “CD4” OR “CD8” (MeSH terms) OR “CD8”
5	“B cell” (MeSH terms) OR “B cell”
6	1 AND 2 AND 3 AND 4 AND 5

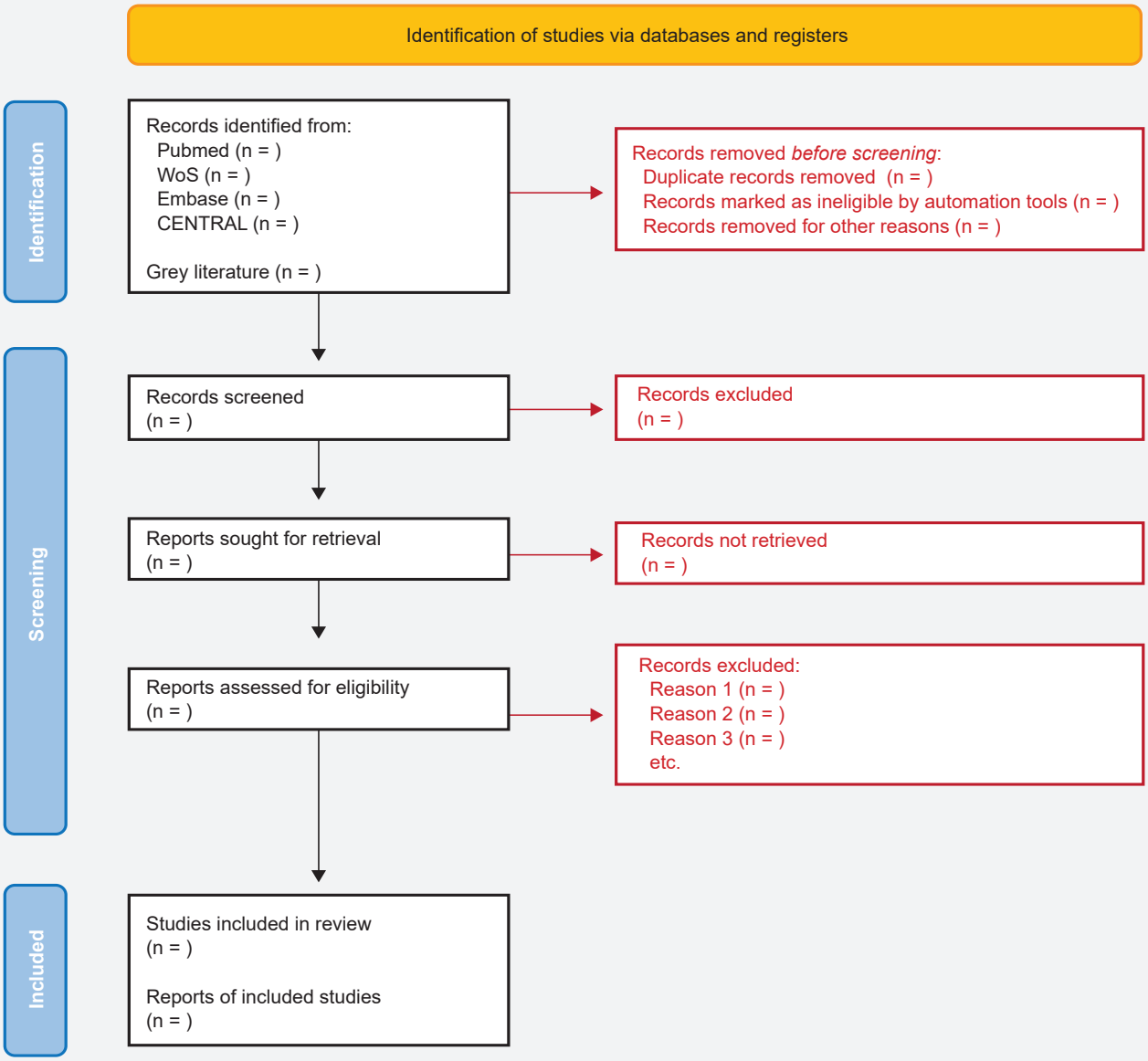

Figure 1 – PRISMA flow diagram of screening process

be categorized according to their quality by two independent reviewers (blind to each other) and disagreements will be solved by discussion or by a third reviewer. The Cochrane Collaboration Tool will be used for the assessment of bias of RCTs. For non-randomized trials the Newcastle-Ottawa Scale (NOS) will be used to assess quality. This is a reliable and valid tool to assess case-control and cohort studies.

Data synthesis

Data from eligible studies will be systematically presented. It will be of interest in this review: patient characteristics, time of blood collection for lymphocyte levels in hospitalized patients with acute pancreatitis (first 24 hours of hospitalization, 48 hours, 72 hours and during the first week), patient outcomes and determination of the severity of acute pancreatitis. The GRADE approach (Grading of Recommendations Assessment, Development and Evaluation) will be used to assess the certainty of the evidence.

Due to the expected heterogeneity of the populations included in the studies, as well as heterogeneity in the timepoints chosen for blood collection and for the different scores of severity, we do not intend to perform a meta-analysis of the collected data. Therefore, the results of the

review will be reported in a table and a narrative synthesis of the findings will be provided with the previously mentioned data.

The three reviewers that will be involved in the selection process, methodological evaluation of the studies and data extraction and synthesis will be the same during the entire process of the systematic review/meta-analysis.

DISCUSSION

Acute pancreatitis is an acute inflammatory process of the pancreas that has a high prevalence rate and varying degrees of severity that can be potentially life threatening. Although there have been significant advances in the understanding of the pathophysiology of acute pancreatitis, much is still unknown about which mechanisms determine its course and severity.[25]

As previously mentioned, The Revised Atlanta Classification of acute pancreatitis is the most widely accepted classification of the severity of acute pancreatitis. Around 70% of patients have mild acute pancreatitis with an uncomplicated course and early discharge from hospital but around 20% - 25% have a moderately severe acute pancreatitis with prolonged hospitalization and around 10% develop severe acute pancreatitis with a significant risk of morbidity and death.[7]

Simpler tools, including risk prediction scores, and preferably easily obtainable biomarkers to predict the severity early in the course of acute pancreatitis are needed. Predicting the severity of this disease as well as knowing its critical mechanisms is essential to better monitor and develop future treatments for patients who need them the most, which may help reduce morbidity and mortality.

The determination of blood lymphocytes, particularly of T cells, has been pointed out as a simple way of determining its severity early in the course of acute pancreatitis and as a future target for the treatment of acute pancreatitis.[26,27] Peripheral blood lymphocyte depletion in acute pancreatitis may result from both excessive apoptosis and migration to the site of inflammation.[28]

Clinical trials have already been conducted such as high-volume hemofiltration treatment in patients with severe acute pancreatitis aimed at ameliorating immune function by removing inflammatory mediators such as TGF-beta1, IL-10, IL-6, and IL-17 and by doing so reducing the imbalance between two groups of T cells, the Th17 and T regulatory cells.[29]

Other clinical trials have included corticosteroids because there is a significant inflammatory response. However, because of patient heterogeneity, the lack of immunological outcomes and the fact that definitions of the severity of acute pancreatitis and treatment protocols are still variable, the findings from these studies are largely exploratory.[30]

Therefore, a better understanding of the role of the cells of the adaptative immune system in this disease might have a huge impact on the outcomes and future treatments of patients with acute pancreatitis not only by trying to increase their number in peripheral blood but also by improving the function of some of the subpopulations of B cells and T cells that are recruited to the inflamed pancreas which most likely are responsible for the production of cytokines and interferons and consequently for the perpetuation of local and systemic inflammation.

To our knowledge, no systematic review outlining the current understanding of blood lymphocytes and severity of acute pancreatitis has been attempted, making our study the first to do so. We expect high heterogeneity between studies concerning the classification of the severity of acute pancreatitis as there is no consensus on which score, or biomarker, is best in predicting acute pancreatitis severity. This is what we believe to be the main limitation of this review.

This report describes the systematic review protocol that will be used to determine the association between peripheral blood B and T lymphocytes and the severity of acute pancreatitis. The results of this study may help to understand the pathophysiology of acute pancreatitis and the role of blood lymphocytes in this disease as well as to identify a new easily applicable and accessible biomarker predictive of the severity of acute pancreatitis.

AUTHOR CONTRIBUTIONS

FM: Study conception and design, drafting of the manuscript.

MN, LMB: Study conception and approval of the final version of the manuscript.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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