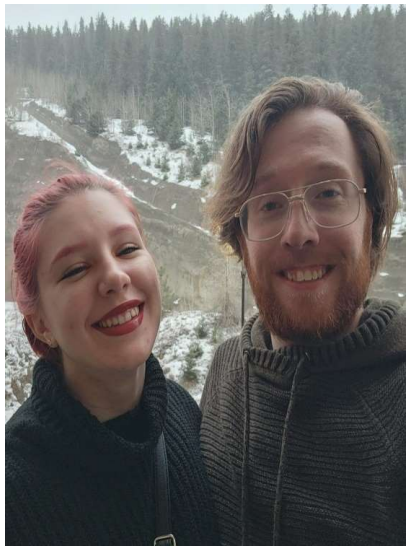
NETs and COVID-19 in Human Blood



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ISCORE FA22
February 3rd, 2023
Anschutz Health Science Building

University of Colorado **Anschutz Medical Campus**

NETs and COVID-19 in Human Blood

By: Ryleh Rapplean
Scholar: Mahmood Kabeil

Introduction

- I am a senior obtaining my bachelor's degree in Biology at University of Colorado - Denver
- I initially joined ISCORE to learn about other cultures and possibly gain some research experience
- I met Mahmood through ISCORE in Fall 2021
- We began working on the systematic review in Spring 2022

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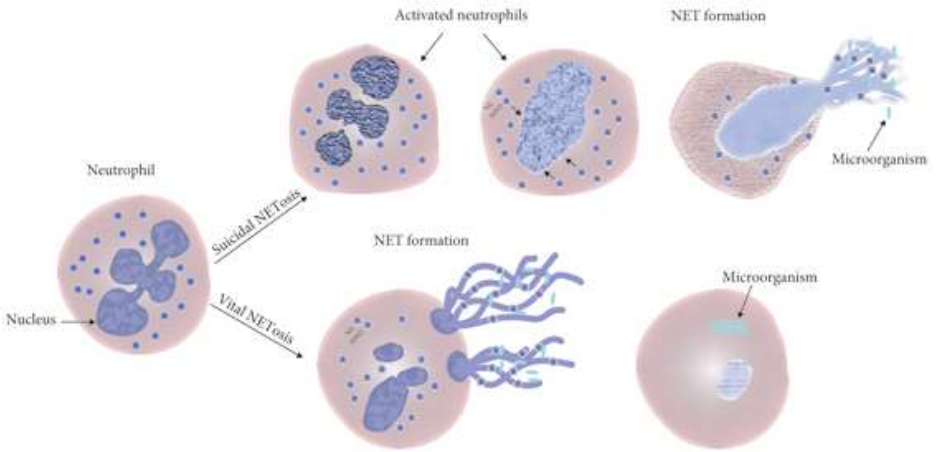
Background

- What are Neutrophil Extracellular Traps (NETs)?
- Initially described in 1996 by Takei, et al. and further described and named by Brinkmann, et al.
- Brinkmann indicated that NETs were made of myeloperoxidase, neutrophil elastase, and DNA, among other components
- In 2011, it was found that certain diseases could induce NETosis and increase the risk for Acute Respiratory Distress Syndrome (ARDS) (3)
- The present review attempts to elucidate the connection between COVID-19 and NETosis using an investigation of the current research



(4)

Methods: Initial Research

- We began our research with a preliminary investigation of relevant terms that could be used to gain papers
- The terms were then inputted into three databases with the guidance of a librarian
- All results were imported into Covidence

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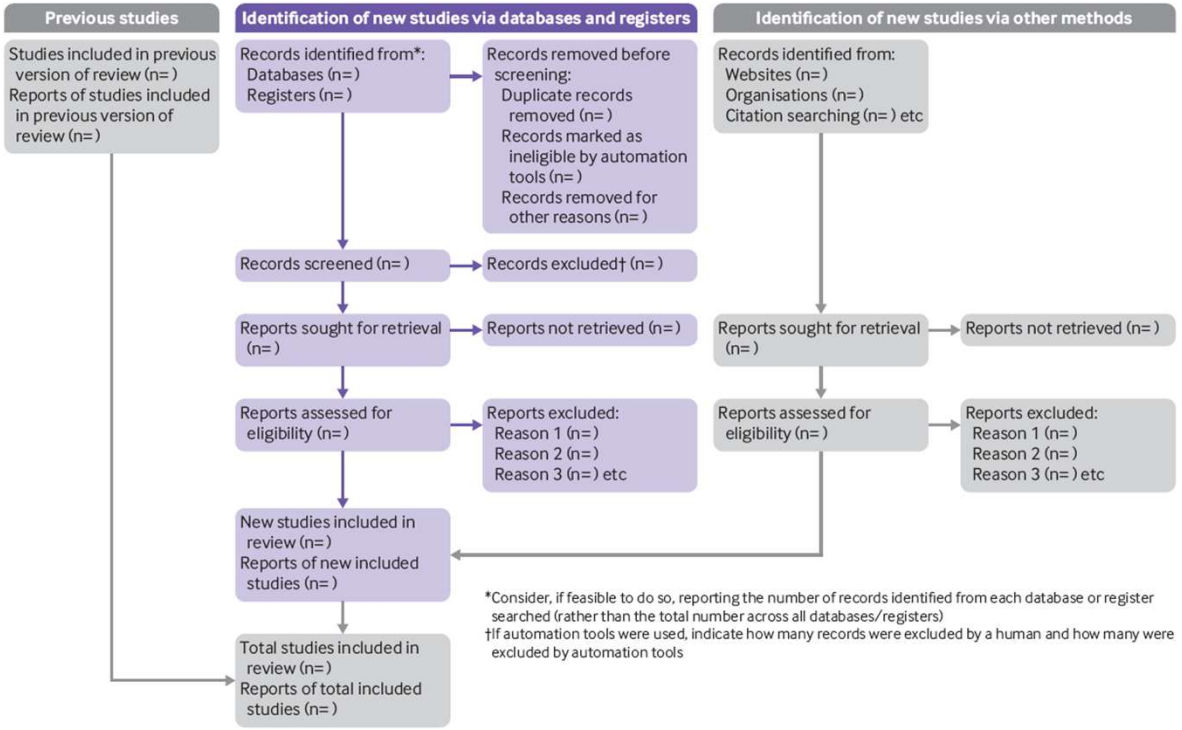
PRISMA Guidelines



(5)

Table 1 | PRISMA 2020 item checklist

Section and topic	Item #	Checklist item	Location where item is reported
Title			
Title	1	Identify the report as a systematic review.	
Abstract			
Abstract	2	See the PRISMA 2020 for Abstracts checklist (table 2).	
Introduction			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
Methods			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesise results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesised results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
Results			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram (see fig 1).	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesised results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
Discussion			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
Other information			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Table 2 | PRISMA 2020 for Abstracts checklist*

Section and topic	Item #	Checklist item
Title		
Title	1	Identify the report as a systematic review.
Background		
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.
Methods		
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.
Synthesis of results	6	Specify the methods used to present and synthesise results.
Results		
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).
Discussion		
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).
Interpretation	10	Provide a general interpretation of the results and important implications.
Other		
Funding	11	Specify the primary source of funding for the review.
Registration	12	Provide the register name and registration number.

*This abstract checklist retains the same items as those included in the PRISMA for Abstracts statement published in 2013,[34] but has been revised to make the wording consistent with the PRISMA 2020 statement and includes a new item recommending authors specify the methods used to present and synthesise results (item #6).

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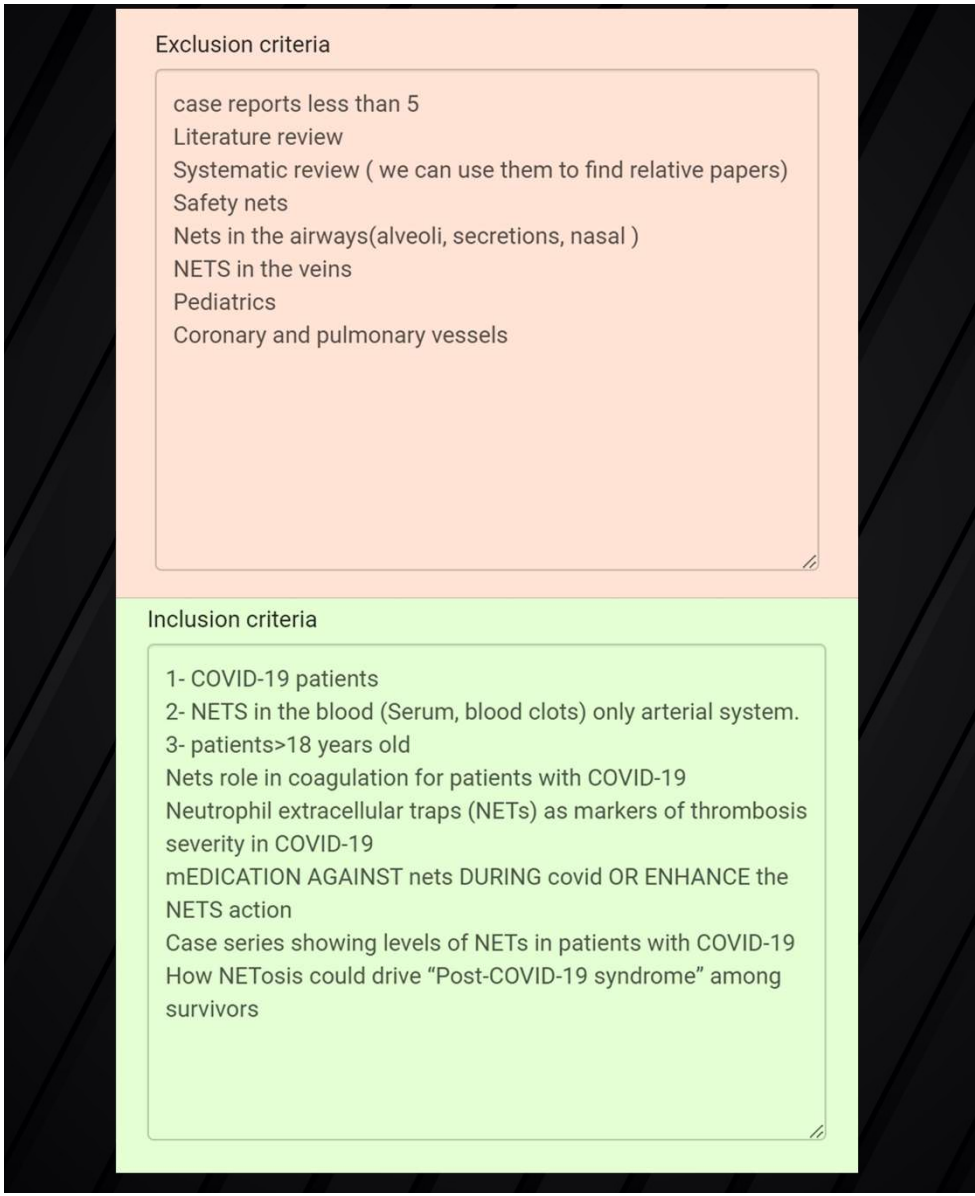
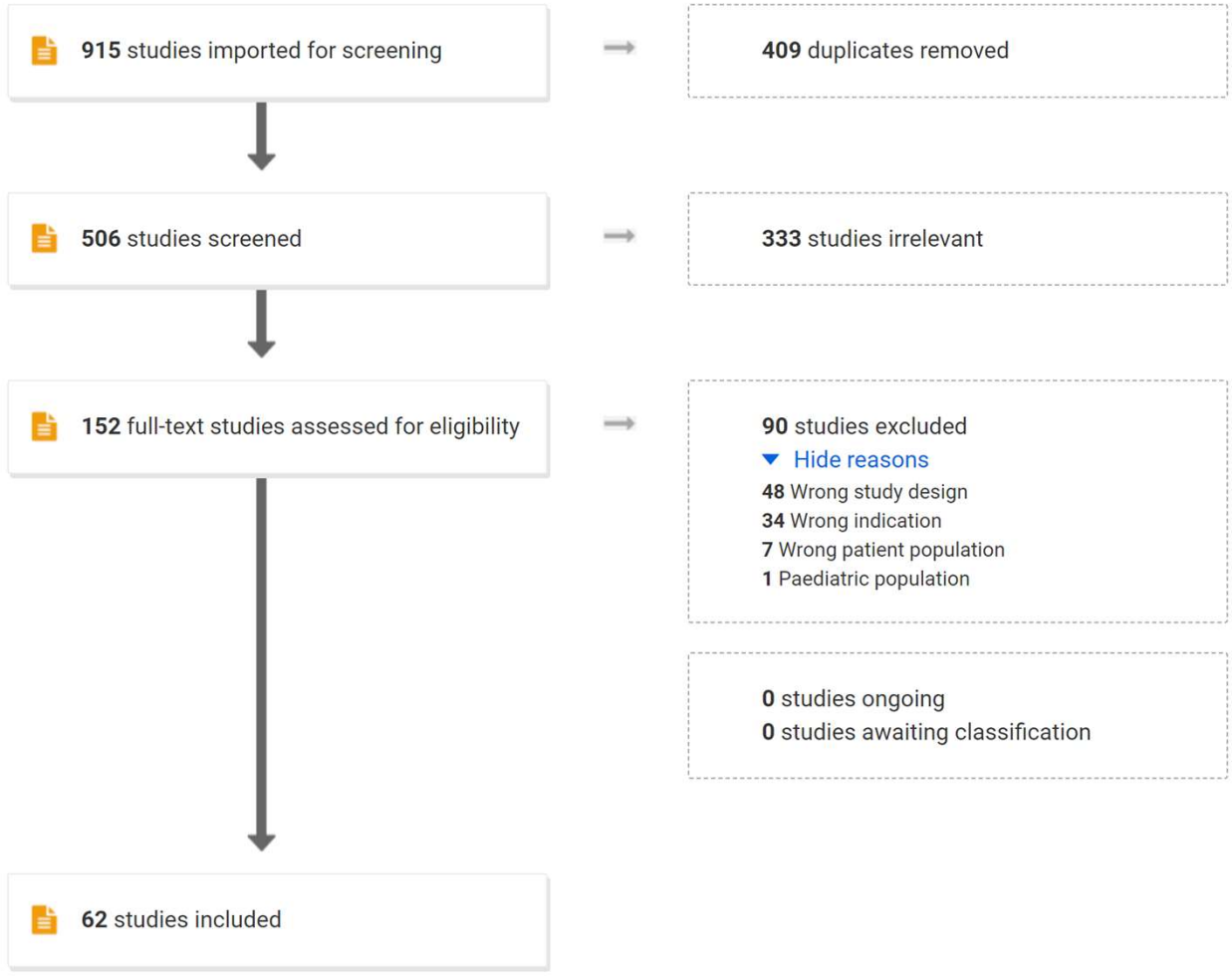
Methods: Covidence

- Formal criteria was created to guide the paper selection process
- Two reviewers read the titles and abstracts of each paper to determine whether it was primary research that matched the criteria
- Papers which were included were then read in full and included or excluded according to the criteria



PRISMA Chart



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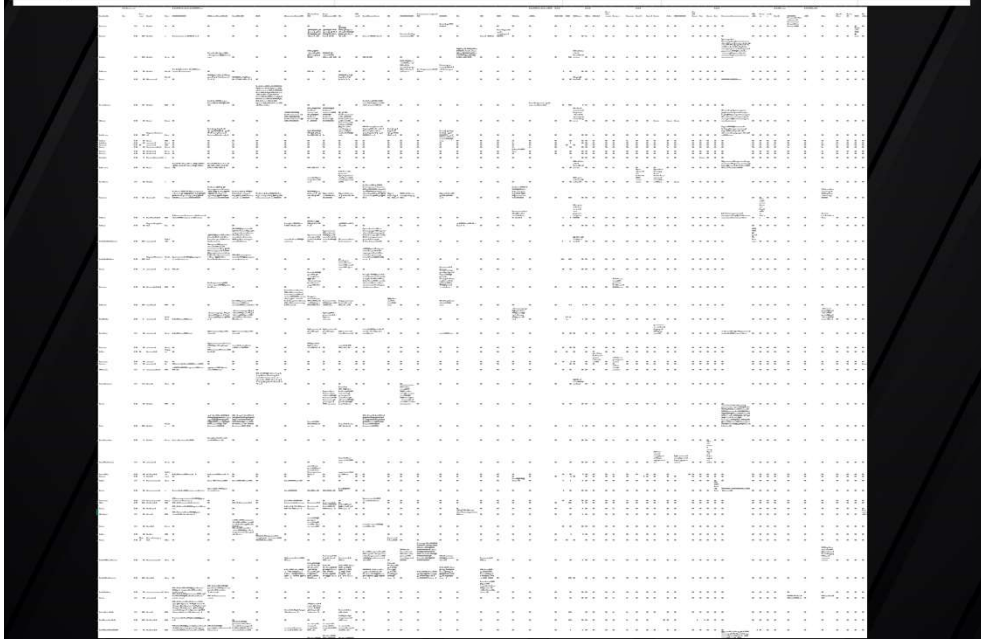
Methods: Data Collection

- After papers were included, they were put into a spreadsheet and specific variables such as basic information, biomarkers, and demographics of cohorts were inputted
- An analytics expert directed us on data cleaning
- For the results today, 18 papers were analyzed for similarities such as the use of certain biomarkers and certain techniques used to quantify NETs

Table I: Basic Information

Papers /Variables	Year	Total # of	Type study	Country
Ouwendijk, et al.	2021	82	Case-control Study	Netherlands
Peyneau, et al.	2022	106	Cross-sectional clinical	France
Skendros, et al.	2020	35	Clinical study	USA
Staats, et al.	2020	97	Clinical Study	Germany
Strich, et al.	2021	14	Cases Series	USA
Tan, et al.	2021	N/A	Treatment Developme	China
Torres-Ruiz & Absalon-Aguilar, et al.	2021	92	Cross-sectional clinical	Mexico
Torres-Ruiz & Perez-Fragoso, et al.	2021	121	Clinical Study	Mexico
Vanderbeke, et al.	2021	92	Case-control study	Belgium
Veras, et al.	2020	53	Case-control study	Brazil



Preliminary Results

- In a collection of 18 papers, there were 1650 patients, with 249 being COVID-19 and in an ICU, being COVID-19 positive with no other distinction, and 450 controls (7-25).
- One paper, Ouwendijk, et al., showed a negative correlation between d-dimer and disease severity(7). However, most papers showed a positive correlation between NET biomarkers and COVID-19 disease severity(8,10-21).
- Four papers indicated that DNAse could be used to inhibit NETosis in COVID-19 Patients(8-11). One paper indicated that inhibiting C3 proteins could result in NETosis inhibition(12).

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Country	Number of Papers (n=19)
Italy	1
Canada	1
USA	6
Belgium	2
Germany; Belgium	1
France	2
South Korea	1
Spain	1
Netherlands	1
Germany	1
Mexico	1
Brazil	1

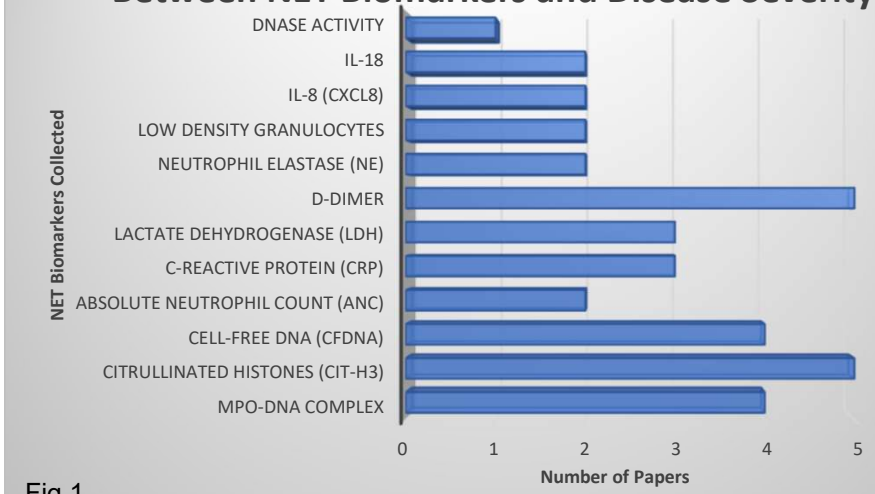

Fig 1.

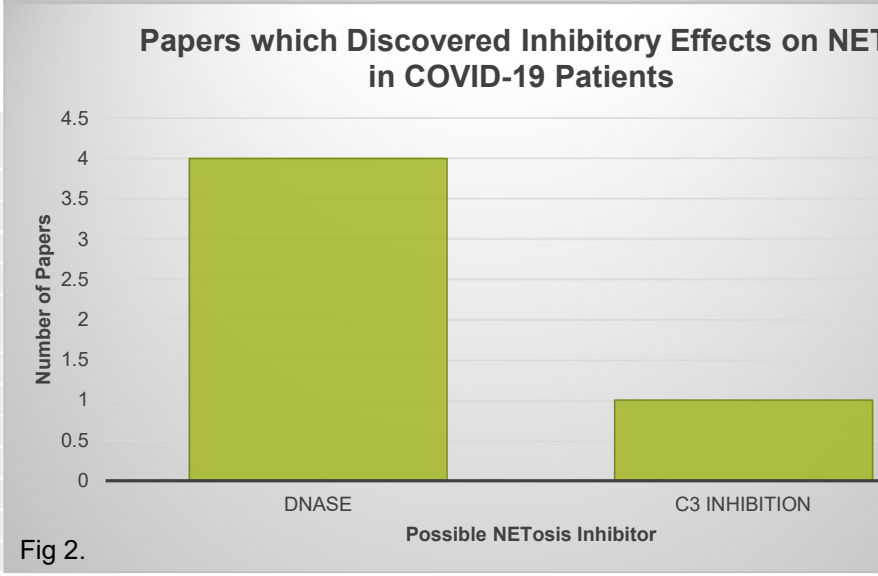

Fig 2.

Preliminary Results

- There was some consensus on NET quantification techniques, with many papers using flow cytometry (7,10,11,13,14,22), but still others using a specific NETosis Assay Kit(13-15).
- Certain papers elected to measure NETs qualitatively rather than quantitatively, indicating only that they had been used as biomarkers(8-13,16,17,22,23,25). This is indicated in figure 4.
- These papers varied in their goals, from quantification of other, less known biomarkers to relating these biomarkers to topics such as defining the maturity of the neutrophils undergoing NETosis(10).

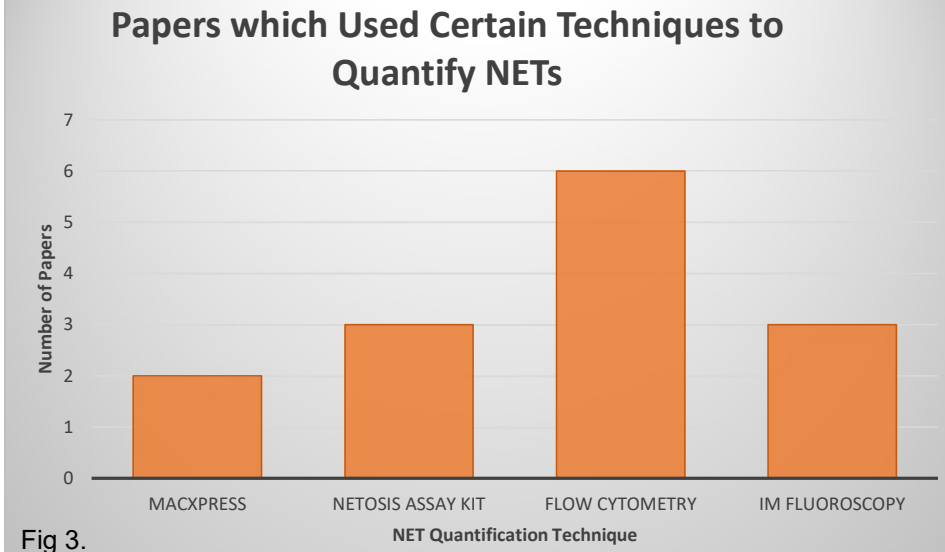

Fig 3.

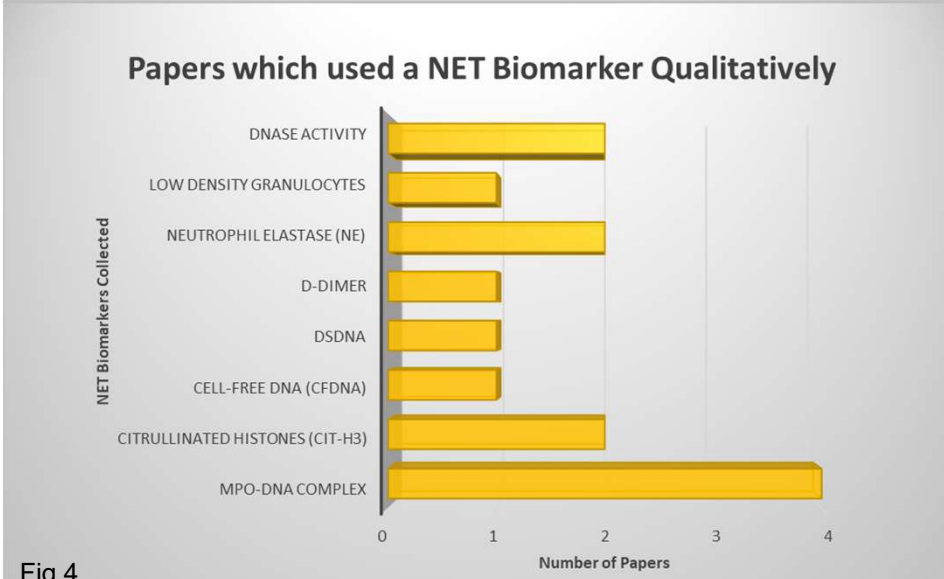

Fig 4.

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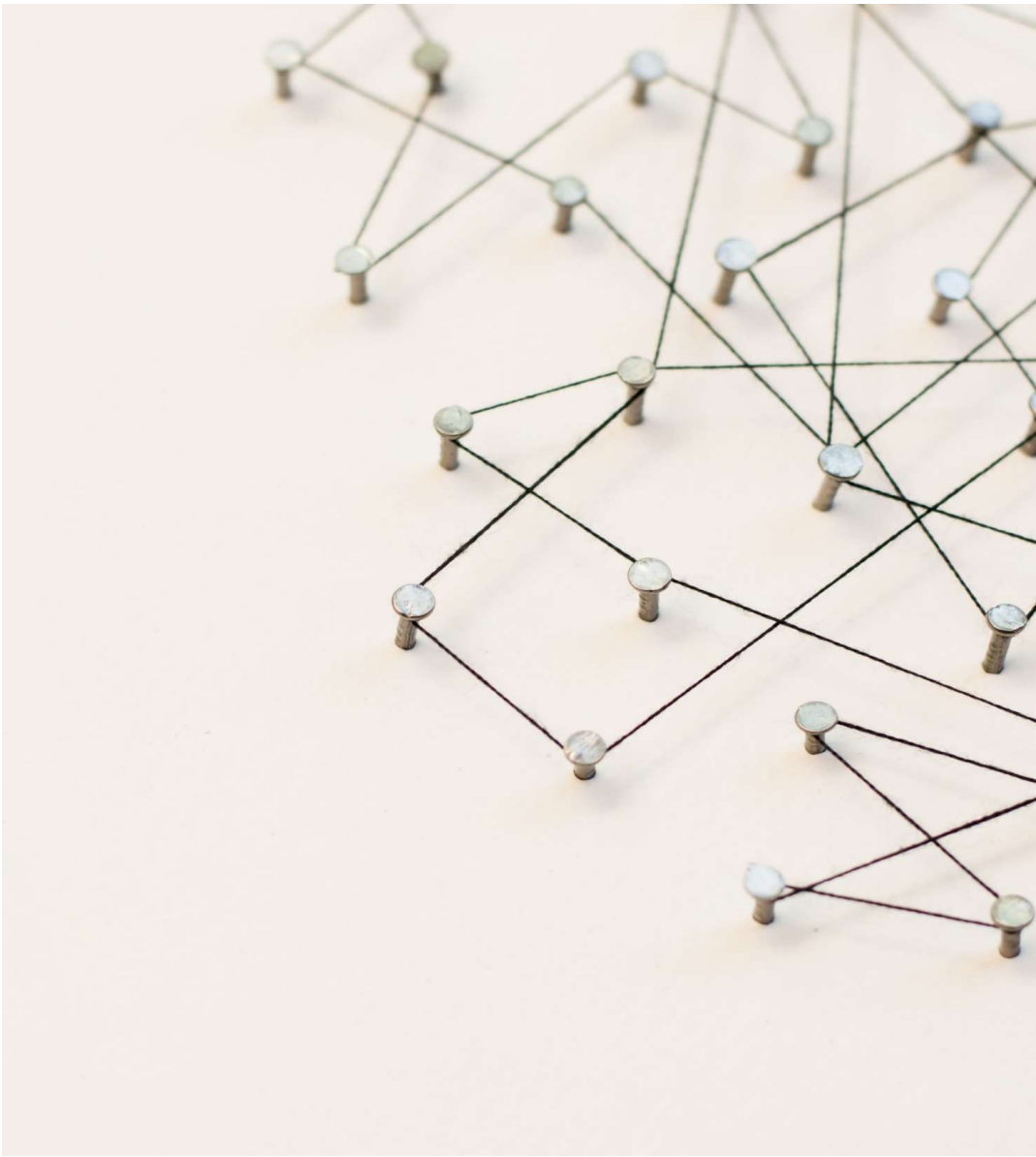
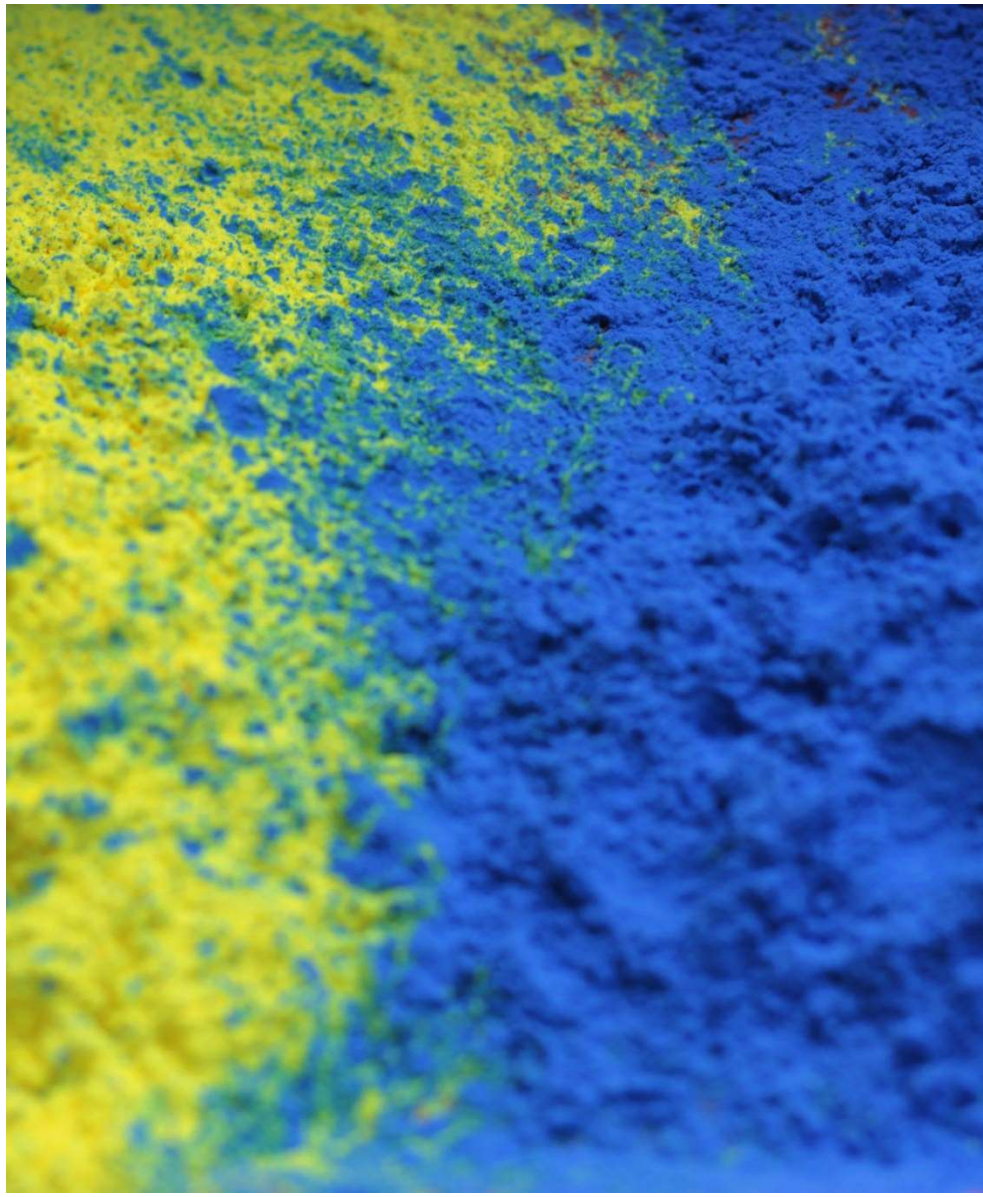
What I Learned

- How to conduct a systematic review
- How to communicate complex information both verbally and in text to a team
- What NETs are and NETs in relation to COVID-19 as well as disease in general
- What “good” data entry looks like and how to “clean” data for analysis



Cultural Takeaways

- Mahmood and I spoke about many differences and similarities between Middle Eastern and US culture
- ISCORE occurred during multiple holidays, we talked about what each person does for those
- Foods were shared, Mahmood made kunafah as well as other foods
- I learned about the culture of Anschutz compared to University of Colorado – Denver and the culture of research as a whole



NETs and COVID-19 in Human Blood

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University of Colorado – Denver[1], University of Colorado Anschutz Medical Campus[2]

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Background

Initially described in 1999 by Takei, et al. and further described and named by Brinkmann, et al. Brinkmann indicated that NETs were made of myeloperoxidase, neutrophil elastase, and DNA, among other components. In 2011, it was found that certain diseases could induce NETosis. The present review attempts to elucidate the connection between COVID-19 and NETosis using an investigation of the current research.

Methods

PubMed, Embase, and Web of Science were the databases used. Search criteria were obtained with the guidance of a librarian. Search results were imported into Covidence, and then screened by two reviewers using a two-step process. The first step screened papers using titles and abstracts, the second involved a more in-depth process of reading the full papers. Papers were included or excluded according to pre-agreed upon criteria. Included papers were then entered into a spreadsheet with the desired variables. The collected data was cleaned, and analysis was conducted to yield the results.

Results

In a collection of 18 papers, there were 1650 patients, with 249 being COVID-19 and in an ICU, being COVID-19 positive with no other distinction, and 450 controls(7-24). This included 5, 9, and 5 papers from 2020, 2021, and 2022, respectively. The papers came from 12 countries, with 5 variations of study design(7-24). Two possible NET inhibitors were found, the use of DNAse(8-11) and C3 inhibition(12). The most common technique used to quantify NETs in the present papers was flow cytometry(7,10,11,13,14,21). Some papers elected to quantify NETs or simply gain a qualitative understanding(8,10-20). The most common biomarker used in relation to NETs is MPO-DNA (7,11,13,21).

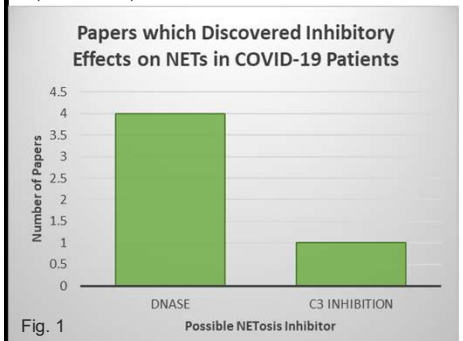

Fig. 1

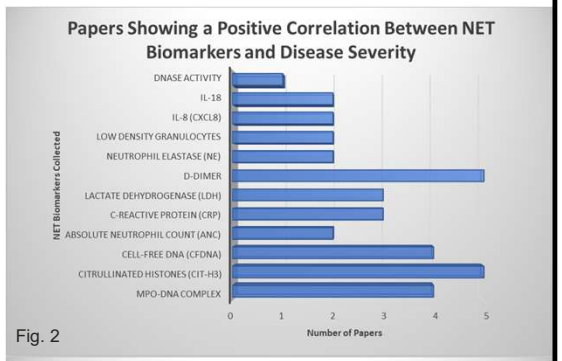

Fig. 2

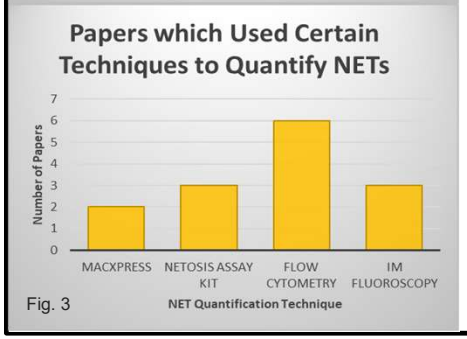

Fig. 3

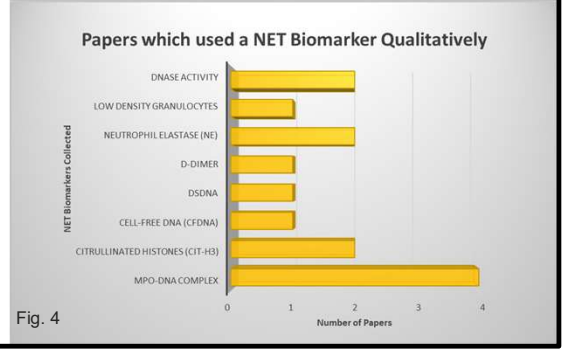

Fig. 4

Conclusions

Although there is some consensus, researchers are using many different biomarkers to collect quantitative and qualitative data. Among the most commonly used are D-dimer, myeloperoxidase-DNA complexes, and citrullinated histones. There seems to be some consistency in methodology for collecting NETs from COVID-19 patients, with flow cytometry being the most common. It is also important to note that some papers defined the presence of NETs while quantitatively measuring other biomarkers. Finally, there is some consensus on the inhibition of erroneous NETs within COVID-19. Four papers indicated that inhibition of DNAse could be used, and one paper indicated C3 inhibition as a possible target as well.

Implications

This study shows a lack of consistency in measuring NETs, possibly because they were only understood to play a role in viral disease beginning in 2011 (3). In the future, more research could be done to determine the most efficient biomarkers which correlate to NETosis and COVID-19 severity, if not disease severity in general.

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