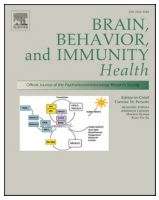
Brain, Behavior, & Immunity - Health 42 (2024) 100904

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx



Why PNI scientists need to engage in exploratory hypothesis-generating biomarker studies

Bianka Karshikoff [a,b]

[a] University of Stavanger, Dept. of Social Studies, Stavanger, Norway
[b] Karolinska Institutet, Dept. of Clinical Neuroscience, Stockholm, Sweden

A B S T R A C T

Multi-omics research is developing rapidly, offering extensive sample analysis options and advanced statistical solutions to identify and understand complex networks of biomarkers. This review encourages groups in the psychoneuroimmunology field with limited experience in *omics* research to embrace these advances. Cross-sectional studies can leverage existing sample collections to provide unique information that complements longitudinal studies, providing insights into which biological systems may warrant further investigation and building fundamental mechanistic knowledge of biological networks. The understanding of immune-brain interactions should inform ongoing developments in exploratory, hypothesis-generating research. Disregarding psychoneuroimmunological aspects may have led to challenges in some prior biomarker research. Moving forward, a more nuanced perspective on inflammation and psychological comorbidity is needed. The first steps in the conceptualization of an explorative cross-sectional *omics* study are discussed from a pragmatic perspective, highlighting who we choose to study and what we choose to measure.

1. Introduction

To expand our knowledge of immune-brain communication, psychoneuroimmunology (PNI) scientists should engage in hypothesis-generating biomarker studies to a larger degree, despite the apparent shortcomings compared to hypothesis-driven research. This can be done using different *omics,* such as genomics, epigenomics, transcriptomics, proteomics, and metabolomics. The term multi-omics refers to a research approach that investigates several *omics* data types, where each type reveals different aspects of a biological system. It is advanced, exploratory biomarker research that maps the biological networks involved in health and disease. (Multi)-omics is an essential part of personalized/precision medicine, where individual differences are understood on a molecular level so that medical treatment can be tailored to each body. For PNI research, *precision health* may be a more useful term, which includes precision medicine, but also disease prevention and health promotion - fields of particular importance for the PNI community. (https://www.cdc.gov/genomics/about/precision_med.htm; accessed 240216).

The goal is usually to identify a set or network of biomarkers, i.e. a biomarker signature (Davis et al., 2020; Gomez-Varela et al., 2019). It should be noted that the term biomarker can encompass brain imaging data and physiological characteristics as well (Davis et al., 2020), but in this review, the term refers to biological sample markers. The terms biomarker and biomarker signature are used interchangeably throughout the review.

Excellent recent reviews give advice on multi-omics studies (Babu and Snyder, 2023; Mengelkoch et al., 2023). These reviews encourage longitudinal designs and highlight the many intriguing possibilities such studies entail. As these reviews point out, a longitudinal study design is superior for biological data with large internal variation, but may be time-consuming due to longitudinal sampling, logistically challenging with repeated sampling, and costly with the analysis of multiple samples for each participant. Well-thought-through, smaller, cross-sectional studies with deeply phenotyped cohorts may complement such studies (Baskozos, 2023), and are the focus of this review. This review will not discuss in detail sampling issues (Ghafouri et al., 2022; Gonzalez-Dominguez et al., 2020; Wasserfall et al., 2022), analysis techniques or statistical concerns (Babu and Snyder, 2023; Forlin et al., 2023; Geyer et al., 2016; Moqri et al., 2024), as this has been done extensively elsewhere. Instead, it will discuss how smaller cross-sectional PNI studies can complement the ongoing multi-omics development. A pragmatic approach is used to focus on the first steps of conceptualization of an exploratory biomarker study. The review is written for colleagues in the PNI field with interesting samples in the freezer from prior studies and assumes a beginner's perspective on *omics*. Our collective understanding of the body-mind interactions is greatly needed in the growing multi-omics field.

E-mail address: bianka.karshikoff@uis.no.

https://doi.org/10.1016/j.bbih.2024.100904
Received 16 February 2024; Received in revised form 4 November 2024; Accepted 8 November 2024
Available online 9 November 2024

2. Current trends in the *omics* field

Multi-omics research has increased explosively, now being part of many major funding initiatives (e.g., https://www.nih.gov/news-events/news-releases/nih-awards-503-million-multi-omics-research-human-health-disease; accessed 240216). The calls are highly ambitious, requiring consortia and longitudinal data collections that take years. The analysis costs for such large, longitudinal multi-omics studies ought to be substantial, and hopefully, in return, results will advance our knowledge substantially. An inspiring example, representative of current trends in the field, is the A2CPS initiative (Berardi et al., 2022), an ambitious project that will undoubtedly advance chronic pain research. A painful event (surgery) serves as the baseline, and patients are followed up to see if they develop post-surgical chronic pain. A wide array of risk and resilience factors are assessed, survey and tissue-based, using a multi-omics approach to identify novel biomarkers for chronic pain. An elegant design aspect of the study is that patients undergoing knee-replacement surgery, who often already experience chronic pain before surgery, can be compared to patients undergoing thoracic surgery, who usually do not have chronic pain. This way, the development from acute to chronic pain can be studied in relation to prior pain. However, no matter how many biomarkers are measured in the most sophisticated way, this study provides insights into mechanisms primarily related to post-surgical chronic pain within the first three months of surgery. The findings may thus not apply to all types of pain or to long-term chronification mechanisms. This is not to diminish this significant effort but to exemplify how the interpretation of identified markers will always be limited by design choices. While initiatives like these are essential, they require extensive resources and take years to complete. It can be argued that there is a need for smaller, cross-sectional studies as well, that perhaps encompass unusual samples, such as suitable experimental stimulations or a combination of animal and human data that reveal novel mechanisms, networks and pathways. They will require less resources and time and can still provide unique findings that can inform larger longitudinal studies.

2.1. Technical developments in the omics field

Multi-omics research is developing on several levels at once. Recent advances in high-throughput molecular analysis techniques enable *omics* measurements ranging from liquid tissue like blood, to investigations of certain cells in detail (single-cell analysis), depending on the research question. For proteomics, for example, elegant solutions for targeted analyses have been developed that enable the simultaneous measurement of several thousands of known peptides. They resemble traditional antibody-based tests but on a much larger scale, enabled by innovative techniques for molecule recognition (Sun et al., 2018, 2023). Please see prior reviews for more information on targeted and untargeted tissue analysis techniques (Babu and Snyder, 2023; Fu et al., 2023; Mengelkoch et al., 2023; Sherrod and McLean, 2016; Vandereyken et al., 2023). Practically all types of samples can be analyzed - even dried blood spots, a very convenient sampling form that can be collected from home (Fredolini et al., 2024). To analyze multi-omics datasets, statistical techniques with machine learning and artificial intelligence components are being developed (Mengelkoch et al., 2023; Vahabi and Michailidis, 2022). One significant challenge here is integrating different data types in the analysis, and several recent reviews have been published that discuss this critical issue (Athieniti and Spyrou, 2023; Sathyanarayanan et al., 2023; Worheide et al., 2021). The analyses can be combined with network analyses that identify biomolecular networks that arise from integrated *omics* data and offer insights into the biological mechanisms that underlie biological processes (Forlin et al., 2023). Each developmental area is a separate research field that is progressing rapidly, which can seem daunting. Although an extensive network of laboratory and biostatistical resources is invaluable, a pragmatic argument can be made for outsourcing the sample and statistical analysis, should such resources not be available. Several companies offer services for both sample analysis and statistical analysis (Sun et al., 2018, 2023). Other companies specialize in bioinformatics data analysis services (e.g.(Hédou et al., 2024; Yaseen et al., 2023)). Despite the impressive advances in the field, it should be acknowledged that there is an intrinsic problem between sample size and the number of variables measured in *omics* studies that need to be considered (see also section 4.2).

3. A PNI perspective on biomarker exploration

Omics studies are thus quite complex due to the various considerations involved and the diverse expertise required. Recent reviews (Babu and Snyder, 2023; Mengelkoch et al., 2023; Moqri et al., 2024; Sathyanarayanan et al., 2023) may give the impression that this type of research is so intricate that it should only be carried out by large laboratories and consortia with abundant resources. This review attempts to lower the threshold for exploratory studies for PNI scientists. Laboratories with specialized knowledge of certain systems should contribute to the field to ensure that we obtain a comprehensive understanding of how the body works – including the brain, emotions, and behaviors.

3.1. Multi-omics for PNI-researchers

Many PNI scientists who study interactions between psychological processes and immune function by focusing on cytokines may benefit from incorporating a multi-omics approach to their research. The current approach of PNI researchers provides a vital complement to that of many immunological and medical researchers, by ensuring that understanding somatic aspects does not come at the expense of understanding psychological aspects. There are limitations to focusing on cytokines and even networks of cytokines (Åström Reitan et al., 2024). It is well known that immune system activation interacts with multiple systems outside the immune system (e.g. (Chi, 2022; Haykin and Rolls, 2021),), and understanding these interactions cytokine by cytokine, or even within a network of cytokines, is limiting. This approach is unable to reveal critical regulatory functions or unexpected consequences of cytokine activation by focusing on a limited number of markers. Combining several layers of data can instead indicate which systems "move together" and which novel molecules should be investigated further in a structured manner.

Given the complexity of the human body, research findings are, to a large extent, hypothesis-generating even for the most ambitious *omics* studies at this point in scientific development (Forlin et al., 2023; Klein et al., 2023; Tebani et al., 2020). There will always be more potential markers to be studied until we can measure them all. There are, for example, still relatively limited possibilities to study tissue biomarkers in the human central nervous system. Moreover, interpreting the functions of identified biomarker signatures relies on prior knowledge of the molecules and available reference databases. Smaller exploratory studies can provide insights into which biological systems and tissues may warrant further investigation, and studies with experimental components may build fundamental mechanistic knowledge that is helpful in the understanding of biomarker function. This task should be embraced by the PNI community to ensure that our unique understanding of the body and mind is considered in future *omics* studies. Below are some aspects to consider when trying to understand immune-brain-mechanisms using *omics*. The examples mainly depart from chronic pain research, but the principles are transferable to other complex disorders.

3.2. A nuanced understanding of inflammation

The term inflammation is often used in an unnuanced manner. While inflammation is usually viewed negatively due to its association with

various chronic disorders, inflammation can be beneficial for recovery in certain contexts or phases of disease (Parisien et al., 2022). Recent proteomics studies exemplify this complexity well. In a series of studies using a panel of inflammatory markers, several cerebrospinal fluid (CSF) inflammatory markers were identified that are invertedly related to pain severity in chronic back pain disorders (Palada et al., 2020). This suggests that some inflammatory cytokines may, in fact, be part of recovery processes. Other CSF inflammatory markers showed a U-shaped relationship with pain severity (Rosenstrom et al., 2024). This finding signifies the importance of precision health, where several individual differences related to health are considered simultaneously and in interaction. In this example, high and low cytokine levels mean different things in terms of pain symptoms in different people, perhaps due to the progression of pain (see section 3.3) or other individual differences in symptomology (see section 3.4). Furthermore, while systemic inflammation has been identified in most pain states (Åström Reitan et al., 2024), this study shows a hypoinflammatory state in chronic pain for some blood markers (Rosenstrom et al., 2024). While these findings need corroboration, it is hard to see how such insight could be reached efficiently using only a few markers and hypothesis-driven approaches.

Furthermore, inflammation and immunoreactivity are sometimes used interchangeably, overlooking their distinct significance (Koop et al., 2021). Ongoing inflammation (assessed by inflammatory markers in the blood) represents the current state of immune activity, while inflammatory reactivity (stimulated blood measures) represents the innate capacity of the immune system to respond to challenges. Inflammation can be viewed as a state-like aspect of immune function because it reflects the immune system's current activity, while inflammatory reactivity can be seen as a trait-like aspect of immune function, as it represents a characteristic or inherent capability of an individual's immune system to respond to adversity (Benson and Karshikoff, 2023). Understanding both aspects is crucial in studying immune function and its role in health and disease. Recent pain studies suggest that immunoreactivity, assessed by in vitro LPS stimulations of white blood cells, is a better predictor of pain progression than blood cytokine levels (Generaal et al., 2014; Schrepf et al., 2016, 2023). Fortunately, many PNI studies use stimulated blood in vivo or ex vivo (Benson et al., 2023; Karshikoff et al., 2016; Mansson et al., 2022). Using such samples for exploratory analysis could give important insights into the immune systems' regulatory capacities under pressure.

3.3. What, where and when

The effect of a signaling molecule depends on where and when it is secreted. Understanding the compartmentalization and timing of biomarkers is crucial for interpreting their significance in different contexts. Some markers will act locally in a defined body compartment, while some will be transported through the bloodstream and act systemically. Some markers may have distal effects via a secondary system, like nerves or migrating immune cells, highlighting the interconnectedness of the immune system and other physiological systems (Bower and Kuhlman, 2023). Finally, some markers may just "spill out" of their compartment when a system is unbalanced or disrupted (Dalmau Gasull et al., 2024). Therefore, not only the biological function of a biomarker should be considered, but also localization and disease/life progression (i.e. biological timing) (Karshikoff et al., 2019). Precision health development would benefit from a more nuanced approach to biomarker exploration, that takes timing, compartmentalization, and upstream and downstream events into account. For example, a recent study explores the compartmental and temporal dynamics of bacteria in the body in health and during infections, following individuals over a 6-year period (Zhou et al., 2024). The bacterial composition varies among individuals and body sites and changes during infections. Some types of bacteria interact more with cytokines than others depending on the body site, and interestingly, the interaction is affected by insulin resistance. It would be interesting to investigate if these individual yet relatively stable interactions affect subjectively perceived symptoms during sickness (Lasselin et al., 2018, 2020).

3.4. Considering the body-mind-connection

Fundamental to PNI research is the perspective that there are no clear-cut boundaries between the peripheral body, the central nervous system, and the mind (Martucci et al., 2023) and that intercommunication is bidirectional (Pariante, 2015). This perspective is the key to *omics* exploration for complex disorders, i.e., often chronic conditions characterized by multifactorial etiology involving interactions between biological, environmental, and lifestyle factors. Chronic pain is a good example of a complex disorder. Many aspects of chronic pain are transdiagnostic; individuals with chronic pain commonly experience comorbidities such as anxiety, depression, fatigue and disturbed sleep, and biopsychosocial aspects influence the pain development and progression (Mogil, 2020, 2021). When exploring biomarkers for a pain diagnosis, studies often compare the patient group with healthy controls, assuming they study the disorder that way. What they are, in fact, studying is the disorder *plus* every comorbidity that comes with the disorder, along with potential physical inactivity and social stressors that may result from long-term diseases. The markers will reflect variations in sampling and analysis solutions, individual differences, but also transdiagnostic mechanisms and complaints, and variations related to disease progression. A PNI scientist could thus question if an identified biomarker signature for a pain diagnosis identified using a healthy control group as the comparison is a marker for that pain syndrome, or partly a marker for the psychological comorbidity experienced by individuals with this diagnosis, or behavioral adaptions to chronic illness. Recent studies suggest that distinct biomarker signatures may be related to different aspects of pain, such as pain sensitivity, pain intensity, or clinical pain features (Gerdle et al., 2020; Wahlen et al., 2018, 2020). Other biomarker signatures seem to relate to generalizable psychological features of chronic pain (Karshikoff et al., 2023; Wahlen et al., 2018). To complicate the issue further, studies suggest unique inflammatory characteristics for patients experiencing a combination of pain and mood disturbances (Benson and Karshikoff, 2023), suggesting a double-hit situation. Depressed patients with pain comorbidity had higher IL-6 and granulocyte-macrophage colony-stimulating factor levels than pain-free depressed patients (Zhou et al., 2021), and individuals experiencing negative effects combined with pain had higher blood CRP levels than those reporting only one of the symptoms (Graham-Engeland et al., 2022). The study design needs to take such aspects into account, for example, by carefully choosing relevant control groups and case-control matching.

3.5. The meaning of a biomarker

The goal of multi-omics studies is to identify novel biomarkers, but understanding what the biomarkers signify is equally important. In biomarker research, a challenging issue is that of specificity, and careful consideration should be taken during study design regarding what the biomarker should signify. Some recent pain studies can be mentioned as examples. Endometriosis is a disorder that often leads to pelvic pain and is currently often diagnosed by surgery. Several attempts have been made in the last few years to identify diagnostic biomarkers, as the mean time to diagnosis is 8 years (Ghai et al., 2020), but they have yet to be successful (Burghaus et al., 2024). Most studies have investigated a hypothesis-driven limited set of markers. CA-125, for example, has been reported as a single marker that can discriminate endometriosis from healthy women (Goulielmos et al., 2020). The problem is that several other studies have also recommended it as a biomarker of prevalent cervical, endometrial, and ovarian cancer – the very groups that may require distinction from endometriosis patients (Samare-Najaf et al., 2023). A comparison between these patient groups and endometriosis patients, rather than healthy controls, would perhaps be more beneficial

for a diagnostic biomarker. Also, an *omics* approach could indicate new candidate biomarkers. Similarly, pain studies using the Olink Inflammation panel (https://olink.com/products-services/target/inflammation/; accessed 240216) often report higher blood levels of AXIN1, ST1A1, and SIRT2 in the clinical group, and different studies claim it as a marker for the particular condition studied (Karshikoff et al., 2023). The findings could, in fact, just be some methodological aspect of this particular analysis plate, but it could also signify common mechanisms that have not been considered; this remains to be determined. A marker present in many disorders may seem useless, but it can carry essential information, as for the commonly studied markers IL-6 and CRP in PNI research. IL-6 and CRP have been found to be elevated in a wide range of health conditions (Del Giudice and Gangestad, 2018). Such unspecific markers provide valuable information about the presence and magnitude of inflammation in the body and its role in various physiological processes, aiding in diagnosis, prognosis, and understanding of disease mechanisms. However, IL-6 and CRP are not ideal targets for pharmacological interventions due to their pleiotropic effects and potential for severe side effects (Del Giudice and Gangestad, 2018). We need specific markers for precision health that can be targeted in treatment, preferably upstream of an activation cascade to minimize side effects, as well as mechanistic biomarkers. Exploratory *omics* studies can accelerate our knowledge of transdiagnostic mechanisms *and* specific treatment targets.

4. Some aspects to consider in a cross-sectional study design

The COVID-19 pandemic brought about unique cohorts that have been used elegantly to teach us more about immunology (Klein et al., 2023). In a recent cross-sectional study, 5 different cohorts were examined to understand what the immune system does to recover fully after a SARS-CoV-2 infection *versus* developing long COVID. Unvaccinated previously infected individuals, vaccinated individuals who were previously infected or not, and individuals who developed long-covid or not, were compared. Every subject with long COVID was explicitly matched to a control subject to account for demographic variations between groups that may have an impact on the immunological phenotype. The findings point to broad shifts in several parts of the immune system that are involved in long COVID, as well as differences in hormonal and cortisol levels. Most importantly, several of the findings are relevant to other post-viral disorders (Rodriguez and Brodin, 2024). Infections present a clear delineation between before and after the incident, making it easier to study the progression and effects of the infection. Furthermore, the mechanisms are known to be primarily immunological. In contrast, for complex chronic disorders, symptoms can accumulate gradually. The bodily systems involved interact, change and adapt over time (Hashmi et al., 2013; Karshikoff et al., 2019; Liston et al., 2016). Underlying mechanisms may be multifaceted and poorly understood. This complicates biomarker identification. By leveraging existing sample collections and investigating a wide range of factors and pathways without predefined hypotheses, we can take on the challenge within the PNI community.

Most importantly, while the biomarker analysis will be exploratory, the study design should be based on prior knowledge and hypotheses. Here are some suggestions for the first steps in the conceptualization of an explorative cross-sectional *omics* study.

4.1. Defining the purpose of the biomarker

Defining the purpose of the intended biomarker (signature) may help guide further design decisions. The Food and Drug Administration's Biomarkers, Endpoints and other Tools (BEST) (https://www.ncbi.nlm.nih.gov/books/NBK326791/; accessed 240216) resource is a good start for determining the purpose of the biomarker sought after. *Susceptibility/risk biomarkers* indicate a predisposition for developing future disease. *Prognostic biomarkers* indicate risk for disease recurrence or progression, while *predictive biomarkers* indicate whether a person will respond to a treatment (see also https://www.ncbi.nlm.nih.gov/books/NBK402284/). *Monitoring biomarkers* indicate the status of the disease, while *response biomarkers* indicate the biological activity of an experimental agent without necessarily drawing conclusions about efficacy or disease outcome.

4.2. Sample selection

The key for explorative studies is sharing, reusing, and thinking outside the box, but some basic aspects need to be fulfilled for sample sharing and comparison. Comparing stored clinical/human samples requires similar sampling procedures and instructions for the participants regarding food intake, sleep, etc. Samples must also have been processed similarly. Freeze-thaw cycles need to be considered, should parts of the samples have been analyzed already, and storage time due to possible degradation. The same type of tissue/compartment, analyzed with the same analytical platform controlled for batch differences, can be compared, but not across tissues or platforms. An inspiring initiative based on a proteomics platform is the CORAL Consortium (https://olink.com/community/coral; accessed 240727), where scientists studying neurogenerative disorders can work together, as their samples are handled and analyzed similarly and are, therefore, comparable. Do not use too few samples. Different *omics* sample size calculators are available online and are offered by some analysis companies; see also (Mengelkoch et al., 2023). Sample sizes in published studies vary greatly, ranging from optimistic to impressive, and it is crucial to be aware of the drawbacks of a smaller sample size. However, adding samples not as specific as required to increase power may give less interpretable results.

Consider what characteristics the control group(s) require(s). Confounders may not be managed readily due to the challenges involved in the statistical analysis for *omics* data and should be accounted for in the study design as far as possible. For pain, a relevant control could be a disorder that is similar in comorbidities and lifestyle effects but does not hurt. For neurodegenerative disorders, where patients often experience comorbid pain and depression, a selected pain group with depression could be a control group of interest. Furthermore, scientists tend to focus on patient groups that have the most severe symptoms, as they are the ones in most need of effective treatments. However, if we are to understand how to heal a body, we may want to focus more on groups who have recovered or who have gone into remission. Investigating relative health (recovery, remission, coping, etc.) may say more about the disease mechanism than comparing ill-health with the complete absence of adversity. Finally, identify validation cohorts where the new discoveries can be confirmed (Moqri et al., 2024). As with most human science, populations across the world are not studied equally, and this is a significant problem in the developing *omics* field. Findings from a homogenous group or certain parts of the world will not represent the whole of humanity. Validating prior findings in other populations is essential in future research, and varied populations are encouraged during sample selection (Caetano-Silva et al., 2022).

4.3. Biomarker selection

The selection of the *omic(s)* to investigate should be guided by the events occurring within the studied bodily compartment, upstream and downstream processes related to the biological mechanisms of interest, and the regulatory circuits involved. As discussed, there is a multitude of analysis techniques on all biological levels, and some choices may have to be pragmatic, limited by the type of sample available, and the budget. Some analysis techniques may require fresh samples. Cell-based analyses may require larger sample volumes than blood-based analyses. If the goal is to use animal samples to help confirm results (Gomez-Varela et al., 2019), finding corresponding animal analysis options may be a limitation. Antibody-based analyses and corresponding techniques

measure predefined targets, and a set of markers needs to be chosen from various options available for the chosen platform. If possible, combine markers or pathways previously investigated with markers that have not yet been explored but make sense biologically. Some platforms do not measure absolute but relative concentrations, and this is usually not a problem in exploratory studies. If the study group needs to be categorized in, for example, low or high inflammation individuals, a CRP test can be added as an anchor to established reference levels. Starting with a broader analysis and transitioning to a smaller set of markers for validation might be advisable. The key consideration may not be the number of markers chosen—5000, 500, or 50—but rather the novelty, biological relevance, and integration with other informative data types of those markers. Once these aspects are considered, moving forward in collaborative biostatistical networks or with specialized analysis companies may be more feasible.

4.4. Analyzing the data

Multi-omics research requires collaboration to ensure expertise in several fields. As previously stated, collaborating with an experienced laboratory is the optimal solution. However, if this is not an option, experienced analysis companies can provide valuable support for *omics* novices. They may know which markers tend to be significant in most studies and are thus markers of ill-health rather than the studied disorder. They can help address methodological issues and should have expertise in analyzing different types of data sets (Athieniti and Spyrou, 2023; Sathyanarayanan et al., 2023; Worheide et al., 2021). Be mindful and inquisitive of the scientific experience of the company representatives. Some companies offer statistical services because customers ask for them, but their statistical expertise may not match their technical/laboratory expertise. Having a biostatistician join an initial meeting to gauge the company's statistical competence may be helpful for an inexperienced group. Define the statistical analysis plan in detail and what type of output the company should provide. Understanding the data, making correct interpretations and drawing reasonable conclusions is ultimately the responsibility of the research group.

5. Conclusion

Advances in high-throughput molecular analysis techniques and advanced statistical models make hypothesis-generating biomarker studies more feasible, but the exploratory approach requires specific considerations. Cross-sectional *omics* studies can complement longitudinal multi-omics studies in building mechanistic understanding. The PNI perspective presents essential insights into the understanding of health and disease that need to be integrated into the current biomarker exploration, especially for complex disorders. When considering if those precious samples in the freezer could be used for exploratory analyses, PNI laboratories with modest experience in *omics* studies can significantly contribute to the growing and intriguing work of understanding biological networks involved in health and disease by taking the steps outlined in this review. They need to consider what the biomarkers of choice signify, how confounders, comorbidities and sampling may affect a biomarker signature, and appropriate analytical approaches. The work cannot be done without consulting expertise and careful risk considerations. On the other hand, we cannot understand human health and disease without the knowledge of the PNI community.

Author declaration

The author declares no economic interest in any of the companies mentioned in the review or others, and the selection is biased and based on the author's personal and limited experience.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

I would like to acknowledge the valuable feedback provided by the reviewers during the revision stage. This feedback was particularly important when writing as a single author.

Data availability

No data was used for the research described in the article.

References

Åström Reitan, J.L.M., Karshikoff, B., Holmström, L., Lekander, M., Kemani, M.K., Wicksell, R.K., 2024. Associations between sickness behavior, but not inflammatory cytokines, and psychiatric comorbidity in chronic pain. Psychoneuroendocrinology 167, 107094.

Athieniti, E., Spyrou, G.M., 2023. A guide to multi-omics data collection and integration for translational medicine. Comput. Struct. Biotechnol. J. 21, 134–149.

Babu, M., Snyder, M., 2023. Multi-omics profiling for health. Mol. Cell. Proteomics 22, 100561.

Baskozos, G., 2023. Introduction to a special issue on big data and pain. Pain Rep 8, e1115.

Benson, S., Karshikoff, B., 2023. How can experimental endotoxemia contribute to our understanding of pain? A narrative review. Neuroimmunomodulation 30, 250–267.

Benson, S., Labrenz, F., Kotulla, S., Brotte, L., Rodder, P., Tebbe, B., Theysohn, N., Engler, H., Elsenbruch, S., 2023. Amplified gut feelings under inflammation and depressed mood: a randomized fMRI trial on interoceptive pain in healthy volunteers. Brain Behav. Immun. 112, 132–137.

Berardi, G., Frey-Law, L., Sluka, K.A., Bayman, E.O., Coffey, C.S., Ecklund, D., Vance, C. G.T., Dailey, D.L., Burns, J., Buvanendran, A., McCarthy, R.J., Jacobs, J., Zhou, X.J., Wixson, R., Balach, T., Brummett, C.M., Clauw, D., Colquhoun, D., Harte, S.E., Harris, R.E., Williams, D.A., Chang, A.C., Waljee, J., Fisch, K.M., Jepsen, K., Laurent, L.C., Olivier, M., Langefeld, C.D., Howard, T.D., Fiehn, O., Jacobs, J.M., Dakup, P., Qian, W.J., Swensen, A.C., Lokshin, A., Lindquist, M., Caffo, B.S., Crainiceanu, C., Zeger, S., Kahn, A., Wager, T., Taub, M., Ford, J., Sutherland, S.P., Wandner, L.D., 2022. Multi-site observational study to assess biomarkers for susceptibility or resilience to chronic pain: the acute to chronic pain signatures (A2CPS) study protocol. Front. Med. 9, 849214.

Bower, J.E., Kuhlman, K.R., 2023. Psychoneuroimmunology: an introduction to immune-to-brain communication and its implications for clinical psychology. Annu. Rev. Clin. Psychol. 19, 331–359.

Burghaus, S., Drazic, P., Wolfler, M., Mechsner, S., Zeppernick, M., Meinhold-Heerlein, I., Mueller, M.D., Rothmund, R., Vigano, P., Becker, C.M., Zondervan, K.T., Beckmann, M.W., Fasching, P.A., Berner-Gatz, S., Grunewald, F.S., Hund, M., Kastner, P., Klammer, M., Laubender, R.P., Wegmeyer, H., Wienhues-Thelen, U.H., Renner, S.P., 2024. Multicenter evaluation of blood-based biomarkers for the detection of endometriosis and adenomyosis: a prospective non-interventional study. Int. J. Gynaecol. 164, 305–314.

Caetano-Silva, M.E., Gonzalez-Ricon, R.J., Chalen, I., Best-Popescu, C.A., Antonson, A. M., Kelley, K.W., 2022. Creating psychoneuroimmunology research networks in Ibero-America. Brain Behav. Immun. Health 26, 100532.

Chi, H., 2022. Immunometabolism at the intersection of metabolic signaling, cell fate, and systems immunology. Cell. Mol. Immunol. 19, 299–302.

Dalmau Gasull, A., Glavan, M., Samawar, S.K.R., Kapupara, K., Kelk, J., Rubio, M., Fumagalli, S., Sorokin, L., Vivien, D., Prinz, M., 2024. The niche matters: origin, function and fate of CNS-associated macrophages during health and disease. Acta Neuropathol. 147, 37.

Davis, K.D., Aghaeepour, N., Ahn, A.H., Angst, M.S., Borsook, D., Brenton, A., Burczynski, M.E., Crean, C., Edwards, R., Gaudilliere, B., Hergenroeder, G.W., Iadarola, M.J., Iyengar, S., Jiang, Y., Kong, J.T., Mackey, S., Saab, C.Y., Sang, C.N., Scholz, J., Segerdahl, M., Tracey, I., Veasley, C., Wang, J., Wager, T.D., Wasan, A.D., Pelleymounter, M.A., 2020. Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. Nat. Rev. Neurol. 16, 381–400.

Del Giudice, M., Gangestad, S.W., 2018. Rethinking IL-6 and CRP: why they are more than inflammatory biomarkers, and why it matters. Brain Behav. Immun. 70, 61–75.

Forlin, R., James, A., Brodin, P., 2023. Making human immune systems more interpretable through systems immunology. Trends Immunol. 44, 577–584.

Fredolini, C., Dodig-Crnković, T., Bendes, A., Dahl, L., Dale, M., Albrecht, V., Mattsson, C., Thomas, C.E., Torinsson Naluai, Å., Gisslen, M., Beck, O., Roxhed, N., Schwenk, J.M., 2024. Proteome profiling of home-sampled dried blood spots reveals proteins of SARS-CoV-2 infections. Commun. Med. 4, 55.

Fu, J., Zhu, F., Xu, C.J., Li, Y., 2023. Metabolomics meets systems immunology. EMBO Rep. 24, e55747.

Generaal, E., Vogelzangs, N., Macfarlane, G.J., Geenen, R., Smit, J.H., Dekker, J., Penninx, B., 2014. Basal inflammation and innate immune response in chronic multisite musculoskeletal pain. Pain 155, 1605–1612.
Gerdle, B., Wahlen, K., Ghafouri, B., 2020. Plasma protein patterns are strongly correlated with pressure pain thresholds in women with chronic widespread pain and in healthy controls-an exploratory case-control study. Medicine (Baltim.) 99, e20497.
Geyer, P.E., Kulak, N.A., Pichler, G., Holdt, L.M., Teupser, D., Mann, M., 2016. Plasma proteome profiling to assess human health and disease. Cell. Sys. 2, 185–195.
Ghafouri, B., Ernberg, M., Andrell, P., Backryd, E., Fisher, M.R., Freund-Levi, Y., Grelz, H., Grabel, O., Karlsten, R., Kosek, E., Lofgren, M., Ringqvist, A., Rudling, K., Stalnacke, B.M., Sorlen, N., Uhlin, K., Westergren, H., Gerdle, B., 2022. Swedish Chronic Pain Biobank: protocol for a multicentre registry and biomarker project. BMJ Open 12, e066834.
Ghai, V., Jan, H., Shakir, F., Haines, P., Kent, A., 2020. Diagnostic delay for superficial and deep endometriosis in the United Kingdom. J. Obstet. Gynaecol. 40, 83–89.
Gomez-Varela, D., Barry, A.M., Schmidt, M., 2019. Proteome-based systems biology in chronic pain. J. Proteonomics 190, 1–11.
Gonzalez-Dominguez, R., Gonzalez-Dominguez, A., Sayago, A., Fernandez-Recamales, A., 2020. Recommendations and best practices for standardizing the pre-analytical processing of blood and urine samples in metabolomics. Metabolites 10.
Goulielmos, G.N., Matalliotakis, M., Matalliotaki, C., Eliopoulos, E., Matalliotakis, I., Zervou, M.I., 2020. Endometriosis research in the -omics era. Gene 741, 144545.
Graham-Engeland, J., DeMeo, N.N., Jones, D.R., Mathur, A., Smyth, J.M., Sliwinski, M.J., McGrady, M.E., Lipton, R.B., Katz, M.J., Engeland, C.G., 2022. Individuals with both higher recent negative affect and physical pain have higher levels of C-reactive protein. Brain Behav. Immun. Health 21, 100431.
Hashmi, J.A., Baliki, M.N., Huang, L., Baria, A.T., Torbey, S., Hermann, K.M., Schnitzer, T.J., Apkarian, A.V., 2013. Shape shifting pain: chronification of back pain shifts brain representation from nociceptive to emotional circuits. Brain 136, 2751–2768.
Haykin, H., Rolls, A., 2021. The neuroimmune response during stress: a physiological perspective. Immunity 54, 1933–1947.
Hédou, J., Marić, I., Bellan, G., Einhaus, J., Gaudillière, D.K., Ladant, F.X., Verdonk, F., Stelzer, I.A., Feyaerts, D., Tsai, A.S., Ganio, E.A., Sabayev, M., Gillard, J., Amar, J., Cambriel, A., Oskotsky, T.T., Roldan, A., Golob, J.L., Sirota, M., Bonham, T.A., Sato, M., Diop, M., Durand, X., Angst, M.S., Stevenson, D.K., Aghaeepour, N., Montanari, A., Gaudillière, B., 2024. Discovery of sparse, reliable omic biomarkers with Stabl. Nat. Biotechnol. 42, 1581–1593.
Karshikoff, B., Jensen, K.B., Kosek, E., Kalpouzos, G., Soop, A., Ingvar, M., Olgart Hoglund, C., Lekander, M., Axelsson, J., 2016. Why sickness hurts: a central mechanism for pain induced by peripheral inflammation. Brain Behav. Immun. 57, 38–46.
Karshikoff, B., Tadros, M.A., Mackey, S., Zouikr, I., 2019. Neuroimmune modulation of pain across the developmental spectrum. Curr. Opin. Behav. Sci. 28, 85–92.
Karshikoff, B., Wahlen, K., Astrom, J., Lekander, M., Holmstrom, L., Wicksell, R.K., 2023. Inflammatory blood signature related to common psychological comorbidity in chronic pain. Biomedicines 11.
Klein, J., Wood, J., Jaycox, J.R., Dhodapkar, R.M., Lu, P., Gehlhausen, J.R., Tabachnikova, A., Greene, K., Tabacof, L., Malik, A.A., Silva Monteiro, V., Silva, J., Kamath, K., Zhang, M., Dhal, A., Ott, I.M., Valle, G., Pena-Hernandez, M., Mao, T., Bhattacharjee, B., Takahashi, T., Lucas, C., Song, E., McCarthy, D., Breyman, E., Tosto-Mancuso, J., Dai, Y., Perotti, E., Akduman, K., Tzeng, T.J., Xu, L., Geraghty, A. C., Monje, M., Yildirim, I., Shon, J., Medzhitov, R., Lutchmansingh, D., Possick, J.D., Kaminski, N., Omer, S.B., Krumholz, H.M., Guan, L., Dela Cruz, C.S., van Dijk, D., Ring, A.M., Putrino, D., Iwasaki, A., 2023. Distinguishing features of long COVID identified through immune profiling. Nature 623, 139–148.
Koop, M.A., Lutke Schipholt, I.J., Scholten-Peeters, G.G.M., Coppieters, M.W., 2021. Identifying the most important confounders when assessing the association between low-grade systemic inflammation and musculoskeletal pain: a modified delphi study. Pain Med. 22, 2661–2669.
Lasselin, J., Lekander, M., Axelsson, J., Karshikoff, B., 2018. Sex differences in how inflammation affects behavior: what we can learn from experimental inflammatory models in humans. Front. Neuroendocrinol. 50, 91–106.
Lasselin, J., Schedlowski, M., Karshikoff, B., Engler, H., Lekander, M., Konsman, J.P., 2020. Comparison of bacterial lipopolysaccharide-induced sickness behavior in rodents and humans: relevance for symptoms of anxiety and depression. Neurosci. Biobehav. Rev. 115, 15–24.
Liston, A., Carr, E.J., Linterman, M.A., 2016. Shaping variation in the human immune system. Trends Immunol. 37, 637–646.
Mansson, K.N.T., Lasselin, J., Karshikoff, B., Axelsson, J., Engler, H., Schedlowski, M., Benson, S., Petrovic, P., Lekander, M., 2022. Anterior insula morphology and vulnerability to psychopathology-related symptoms in response to acute inflammation. Brain Behav. Immun. 99, 9–16.
Martucci, K.T., Karshikoff, B., Mackey, S.C., 2023. Links between brain neuroimaging and blood inflammatory markers in urological chronic pelvic pain syndrome. Physiol. Behav. 271, 114358.
Mengelkoch, S., Miryam Schussler-Fiorenza Rose, S., Lautman, Z., Alley, J.C., Roos, L.G., Ehlert, B., Moriarity, D.P., Lancaster, S., Snyder, M.P., Slavich, G.M., 2023. Multi-omics approaches in psychoneuroimmunology and health research: conceptual considerations and methodological recommendations. Brain Behav. Immun. 114, 475–487.
Mogil, J.S., 2020. Qualitative sex differences in pain processing: emerging evidence of a biased literature. Nat. Rev. Neurosci. 21, 353–365.
Mogil, J.S., 2021. Sources of individual differences in pain. Annu. Rev. Neurosci. 44, 1–25.
Moqri, M., Herzog, C., Poganik, J.R., Ying, K., Justice, J.N., Belsky, D.W., Higgins-Chen, A.T., Chen, B.H., Cohen, A.A., Fuellen, G., Hagg, S., Marioni, R.E., Widschwendter, M., Fortney, K., Fedichev, P.O., Zhavoronkov, A., Barzilai, N., Lasky-Su, J., Kiel, D.P., Kennedy, B.K., Cummings, S., Slagboom, P.E., Verdin, E., Maier, A.B., Sebastiano, V., Snyder, M.P., Gladyshev, V.N., Horvath, S., Ferrucci, L., 2024. Validation of biomarkers of aging. Nat. Med. 30, 360–372.
Palada, V., Ahmed, A.S., Freyhult, E., Hugo, A., Kultima, K., Svensson, C.I., Kosek, E., 2020. Elevated inflammatory proteins in cerebrospinal fluid from patients with painful knee osteoarthritis are associated with reduced symptom severity. J. Neuroimmunol. 349, 577391.
Pariante, C.M., 2015. Psychoneuroimmunology or immunopsychiatry? Lancet Psychiatr. 2, 197–199.
Parisien, M., Lima, L.V., Dagostino, C., El-Hachem, N., Drury, G.L., Grant, A.V., Huising, J., Verma, V., Meloto, C.B., Silva, J.R., Dutra, G.G.S., Markova, T., Dang, H., Tessier, P.A., Slade, G.D., Nackley, A.G., Ghasemlou, N., Mogil, J.S., Allegri, M., Diatchenko, L., 2022. Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Sci. Transl. Med. 14, eabj9954.
Rodriguez, L., Brodin, P., 2024. Immune system perturbations in patients with long COVID. Trends Mol. 30, 200–201.
Rosenstrom, A.H.C., Ahmed, A.S., Kultima, K., Freyhult, E., Berg, S., Bersellini Farinotti, A., Palada, V., Svensson, C.I., Kosek, E., 2024. Unraveling the neuroimmune interface in chronic pain-the association between cytokines in the cerebrospinal fluid and pain in patients with lumbar disk herniation or degenerative disk disease. Pain 165, e65–e79.
Samare-Najaf, M., Razavinasab, S.A., Samareh, A., Jamali, N., 2023. Omics-based novel strategies in the diagnosis of endometriosis. Crit. Rev. Clin. Lab. Sci. 1–21.
Sathyanarayanan, A., Mueller, T.T., Ali Moni, M., Schueler, K., members, E.T.N., Baune, B.T., Lio, P., Mehta, D., Baune, B.T., Dierssen, M., Ebert, B., Fabbri, C., Fusar-Poli, P., Gennarelli, M., Harmer, C., Howes, O.D., Janzing, J.G.E., Lio, P., Maron, E., Mehta, D., Minelli, A., Nonell, L., Pisanu, C., Potier, M.C., Rybakowski, F., Serretti, A., Squassina, A., Stacey, D., van Westrhenen, R., Xicota, L., 2023. Multi-omics data integration methods and their applications in psychiatric disorders. Eur. Neuropsychopharmacol 69, 26–46.
Schrepf, A., O'Donnell, M.A., Luo, Y., Bradley, C.S., Kreder, K.J., Lutgendorf, S.K., 2016. Inflammation and symptom change in interstitial cystitis or bladder pain syndrome: a multidisciplinary approach to the study of chronic pelvic pain research network study. Urology 90, 56–61.
Schrepf, A., Kaplan, C., Harris, R.E., Williams, D.A., Clauw, D.J., As-Sanie, S., Till, S., Clemens, J.Q., Rodriguez, L.V., Van Bokhoven, A., Landis, R., Gallop, R., Bradley, C., Naliboff, B., Pontari, M., O'Donnell, M., Luo, Y., Kreder, K., Lutgendorf, S.K., Harte, S.E., See, M.m., 2023. Stimulated whole blood cytokine/chemokine responses are associated with interstitial cystitis/bladder pain syndrome phenotypes and features of nociplastic pain: a multidisciplinary approach to the study of chronic pelvic pain research network study. Pain 164, 1148–1157.
Sherrod, S.D., McLean, J.A., 2016. Systems-wide high-dimensional data acquisition and informatics using structural mass spectrometry strategies. Clin. Chem. 62, 77–83.
Sun, B.B., Chiou, J., Traylor, M., Benner, C., Hsu, Y.H., Richardson, T.G., Surendran, P., Mahajan, A., Robins, C., Vasquez-Grinnell, S.G., Hou, L., Kvikstad, E.M., Burren, O. S., Davitte, J., Ferber, K.L., Gillies, C.E., Hedman, A.K., Hu, S., Lin, T., Mikkilineni, R., Pendergrass, R.K., Pickering, C., Prins, B., Baird, D., Chen, C.Y., Ward, L.D., Deaton, A.M., Welsh, S., Willis, C.M., Lehner, N., Arnold, M., Worheide, M.A., Suhre, K., Kastenmuller, G., Sethi, A., Cule, M., Raj, A., Alnylam Human, G., AstraZeneca Genomics, I., Biogen Biobank, T., Bristol Myers, S., Genentech Human, G., GlaxoSmithKline Genomic, S., Pfizer Integrative, B., Population Analytics of Janssen Data, S., Regeneron Genetics, C., Burkitt-Gray, L., Melamud, E., Black, M.H., Fauman, E.B., Howson, J.M.M., Kang, H.M., McCarthy, M. I., Nioi, P., Petrovski, S., Scott, R.A., Smith, E.N., Szalma, S., Waterworth, D.M., Mitnaul, L.J., Szustakowski, J.D., Gibson, B.W., Miller, M.R., Whelan, C.D., 2023. Plasma proteomic associations with genetics and health in the UK Biobank. Nature 622, 329–338.
Sun, B.B., Maranville, J.C., Peters, J.E., Stacey, D., Staley, J.R., Blackshaw, J., Burgess, S., Jiang, T., Paige, E., Surendran, P., Oliver-Williams, C., Kamat, M.A., Prins, B.P., Wilcox, S.K., Zimmerman, E.S., Chi, A., Bansal, N., Spain, S.L., Wood, A.M., Morrell, N.W., Bradley, J.R., Janjic, N., Roberts, D.J., Ouwehand, W.H., Todd, J.A., Soranzo, N., Suhre, K., Paul, D.S., Fox, C.S., Plenge, R.M., Danesh, J., Runz, H., Butterworth, A.S., 2018. Genomic atlas of the human plasma proteome. Nature 558, 73–79.
Tebani, A., Gummesson, A., Zhong, W., Koistinen, I.S., Lakshmikanth, T., Olsson, L.M., Boulund, F., Neiman, M., Stenlund, H., Hellstrom, C., Karlsson, M.J., Arif, M., Dodig-Crnkovic, T., Mardinoglu, A., Lee, S., Zhang, C., Chen, Y., Olin, A., Mikes, J., Danielsson, H., von Feilitzen, K., Jansson, P.A., Angeras, O., Huss, M., Kjellqvist, S., Odeberg, J., Edfors, F., Tremaroli, V., Forsstrom, B., Schwenk, J.M., Nilsson, P., Moritz, T., Backhed, F., Engstrand, L., Brodin, P., Bergstrom, G., Uhlen, M., Fagerberg, L., 2020. Integration of molecular profiles in a longitudinal wellness profiling cohort. Nat. Commun. 11, 4487.
Vahabi, N., Michailidis, G., 2022. Unsupervised multi-omics data integration methods: a comprehensive review. Front. Genet. 13, 854752.
Vandereyken, K., Sifrim, A., Thienpont, B., Voet, T., 2023. Methods and applications for single-cell and spatial multi-omics. Nat. Rev. Genet. 24, 494–515.
Wahlen, K., Ernberg, M., Kosek, E., Mannerkorpi, K., Gerdle, B., Ghafouri, B., 2020. Significant correlation between plasma proteome profile and pain intensity, sensitivity, and psychological distress in women with fibromyalgia. Sci. Rep. 10, 12508.
Wahlen, K., Ghafouri, B., Ghafouri, N., Gerdle, B., 2018. Plasma protein pattern correlates with pain intensity and psychological distress in women with chronic widespread pain. Front. Psychol. 9, 2400.

Wasserfall, C., Dyer, A.M., Speake, C., Andersen, D.K., Baab, K.T., Bellin, M.D., Broach, J. R., Campbell-Thompson, M., Chinchilli, V.M., Lee, P.J., Park, W.G., Pratley, R.E., Saloman, J.L., Sims, E.K., Tang, G., Yadav, D., Yazici, C., Conwell, D.L., 2022. Standard operating procedures for biospecimen collection, processing, and storage: from the type 1 diabetes in acute pancreatitis Consortium. In: Acute Pancreatitis, C. (Ed.), Pancreas 51, 593–597. Type 1 Diabetes.

Worheide, M.A., Krumsiek, J., Kastenmuller, G., Arnold, M., 2021. Multi-omics integration in biomedical research - a metabolomics-centric review. Anal. Chim. Acta 1141, 144–162.

Yaseen, N.R., Barnes, C.L.K., Sun, L., Takeda, A., Rice, J.P., 2023. Genetics of vegetarianism: a genome-wide association study. PLoS One 18, e0291305.

Zhou, X., Shen, X., Johnson, J.S., Spakowicz, D.J., Agnello, M., Zhou, W., Avina, M., Honkala, A., Chleilat, F., Chen, S.J., Cha, K., Leopold, S., Zhu, C., Chen, L., Lyu, L., Hornburg, D., Wu, S., Zhang, X., Jiang, C., Jiang, L., Jiang, L., Jian, R., Brooks, A.W., Wang, M., Contrepois, K., Gao, P., Rose, S.M.S., Tran, T.D.B., Nguyen, H., Celli, A., Hong, B.Y., Bautista, E.J., Dorsett, Y., Kavathas, P.B., Zhou, Y., Sodergren, E., Weinstock, G.M., Snyder, M.P., 2024. Longitudinal profiling of the microbiome at four body sites reveals core stability and individualized dynamics during health and disease. Cell Host Microbe 32, 506–526 e509.

Zhou, Y., Wang, C., Lan, X., Li, H., Chao, Z., Ning, Y., 2021. Plasma inflammatory cytokines and treatment-resistant depression with comorbid pain: improvement by ketamine. J. Neuroinflammation 18, 200.

Web pages

https://www.cdc.gov/genomics/about/precision_med.htm ; accessed 240216.

https://www.nih.gov/news-events/news-releases/nih-awards-503-million-multi-omics-research-human-health-disease ; accessed 240216.

https://olink.com/products-services/target/inflammation/; accessed 240216.

https://www.ncbi.nlm.nih.gov/books/NBK326791/; accessed 240216.

https://olink.com/community/coral; accessed 240727.