Literature review and methodological considerations for understanding circulating risk biomarkers following trauma exposure

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Abstract

Exposure to traumatic events is common. While many individuals recover following trauma exposure, a substantial subset develop adverse posttraumatic neuropsychiatric sequelae (APNS) such as posttraumatic stress, major depression, and regional or widespread chronic musculoskeletal pain. APNS cause substantial burden to the individual and to society, causing functional impairment and physical disability, risk for suicide, lost workdays, and increased health care costs. Contemporary treatment is limited by an inability to identify individuals at high risk of APNS in the immediate aftermath of trauma, and an inability to identify optimal treatments for individual patients. Our purpose is to provide a comprehensive review describing candidate blood-based biomarkers that may help to identify those at high risk of APNS and/or guide individual intervention decision-making. Such blood-based biomarkers include circulating biological factors such as hormones, proteins, immune molecules, neuropeptides, neurotransmitters, mRNA and noncoding RNA expression signatures, while we do not review genetic and epigenetic biomarkers due to other recent reviews of this topic. The current state of the literature on circulating risk biomarkers of APNS is summarized, and key considerations and challenges for their discovery and translation are discussed. We also describe the AURORA study, a specific example of current

scientific efforts to identify such circulating risk biomarkers and the largest study to date focused on identifying risk and prognostic factors in the aftermath of trauma exposure.

Introduction

Exposure to traumatic events is common[2, 3]. While many individuals recover following trauma exposure, a substantial subset develop adverse posttraumatic neuropsychiatric sequelae (APNS). APNS, as previously defined[4], can include a wide range of neuropsychiatric outcomes; three of the most common APNS are posttraumatic stress (PTS), major depression, and regional or widespread chronic musculoskeletal pain (CMP). These APNS cause tremendous suffering, functional impairment, high health care costs, and are a leading cause of disability among current and former members of the armed forces.[5–14]

Individuals who develop APNS often present for emergency care or other health care in the immediate/early aftermath of the inciting event, creating the opportunity to initiate secondary preventive interventions to those at high risk. Risk stratification tools are applied to tens of thousands of trauma survivors in Emergency Departments throughout the US every day, including both clinician assessments (e.g., CCR and NEXUS[17] criteria for cervical fracture risk) and blood-based biomarkers (e.g., lactate[18–20], base deficit[28, 29]). Unfortunately, no risk prediction tools are in common use that identify trauma survivors at risk of APNS. Such tools could potentially identify at-risk individuals during an early neuroplastic period, when APNS treatments might be most efficacious.[22–24, 30]

Several clinical prediction tools for PTS have been developed using sociodemographic and self-report data (e.g.[31–40]). A number of previous studies have also examined the role of genetic and biological factors on APNS risk[41–51], overall suggesting that biomarkers may improve risk stratification and/or treatment selection vs. clinical and self-report factors alone. Additionally, identification of blood-based risk biomarkers can provide insight into neurobiolgic changes occurring in the early aftermath of trauma exposure that mediate APNS and could identify novel targets for preventative interventions. The purpose of this review is to summarize current literature regarding circulating risk biomarkers of APNS, as well as to examine key considerations and challenges for their discovery and translation. We will also highlight the potential of the AURORA study, a large-scale longitudinal study of trauma survivors that seeks to identify such circulating risk biomarkers.

Circulating biomarkers

The NIH Biomarkers Definitions Working Group defines biomarkers as "characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"[52]. Continued intensive interest in biomarker identification, together with increasing ambiguity regarding the nomenclature for biomarker subtypes, led the FDA and NIH to develop the

Biomarkers, EndpointS and other Tools (BEST) resource. This initiative seeks to improve communication, align expectations, and improve scientific understanding of biomarkers and created definitions for diagnostic biomarkers and susceptibility ("risk") biomarkers.

Diagnostic biomarkers are defined as those that detect or confirm the presence of a disease or condition.[52] Such biomarkers are the most common type of blood-based biological markers identified for APNS to-date[53–55], and include circulating biomarkers cataloged in the PTSD Biomarker Database.[56] Risk biomarkers are defined as biomarkers that indicate the potential for developing a disease that is not yet clinically apparant. Examples of circulating blood-based biomarkers that have been assessed via previous research as risk factors for APNS are displayed in Figure 1. Of note, genetic polymorphisms and epigenetic factors are also promising biomarkers of APNS, however, these will not be reviewed here as a number of review articles have recently covered this topic[57–66].

Summary of current literature

In this review, we surveyed all literature published in NCBI PubMed since its inception and through the end of 2018, using the search strategy provided in Supplementary Figure 1. This search retrieved longitudinal human studies that examined blood-based, circulating biomarkers collected before or proximally to (soon after) trauma exposure that predicted three of the most common APNS outcomes (PTS, depression, and regional or widespread pain) over time. While APNS includes other sequelae (e.g. substance abuse and somatic ("post-concussive") symptoms), they are not included in this review. In particular, and given our focus on circulating biomarkers, our search strategy *a priori* selected original research studies that assess hormones, proteins, cell-free DNA (cfDNA), mRNA and non-coding RNAs, immune markers, neuropeptides, and neurotransmitters, and (as noted above) it excluded studies that examined genotypes and epigenetic modifications. A large number of studies have assessed trauma exposure, APNS outcomes, and circulating biomarkers either retrospectively or cross-sectionally; while these studies examine the diagnostic value of putative biomarkers for APNS, they do not determine whether such biomarkers predict risk for APNS outcomes over time. Thus, such biomarkers were excluded from the present review. All articles retrieved were assessed for their appropriateness by two independent raters (SDL and ASZ), and appropriate results were supplemented with any manually identified articles.

Longitudinal studies to date that examine whether circulating biomarkers predict susceptibility or risk for APNS outcomes over time are summarized in Table 1. The majority of this work has focused on PTS symptoms[44, 67–81] but studies have also examined depressive symptoms[73, 74, 76] and CMP[75, 82–85] as primary phenotypic endpoints. Given the well-established role of the hypothalamic-pituitary adrenal axis (HPA) and glucocorticoid signaling in stress-related disease,[86, 87] many studies to date have examined various components of glucocorticoid signaling, including cortisol levels, glucocorticoid sensitivity, glucocorticoid receptor abundance, cortisol awakening response, and mRNA levels of notable glucocorticoid-responsive genes (e.g., *FKBP5*, *GILZ*, and *SGK1*) in whole blood or peripheral blood monocytes (PBMC).[68, 69, 71, 73, 74] Other studies have examined genome-wide mRNA expression in PBMC or leukocytes,[67, 70, 88] microRNA levels and mRNA

expression of X chromosome gene transcripts in whole blood,[75, 82] cell-free DNA,[83] Vitamin D[85], C-reactive protein (CRP),[80, 83] proinflammatory cytokines,[44, 78, 89] norepinephrine[90], and neuropeptides and neurotransmitters including oxytocin, vasopressin, GABA, and neuropeptide Y in either serum or plasma.[72, 76, 77, 81] While several (but not all) of these studies have found significant associations between biological markers and longitudinal APNS outcomes, to our knowledge no susceptibility/risk biomarkers have been replicated across research groups. Two studies, however, did observe independent replication across two trauma cohorts[84, 88].

Furthermore, and as shown in Table 1 and discussed in the next section, there is considerable heterogeneity in terms of not only the type of biomarker studied but also in the methodololgical details, the phenotypic outcomes, and the characteristics of trauma exposure examined across studies, rendering cross-study comparisons challenging. Importantly, the type, intensity, duration, and timing of trauma are all likely to involve distinct biological responses and molecular changes potentially emerging at different timepoints following trauma exposure. Disentangling the mechanisms of pleiotropic APNS will be a critical task for future research endeavors and a major contribution towards personalized medicine and psychiatry.

One interesting observation from our literature search is that the risk biomarkers assessed included both those biomolecules that are predominantly considered peripherally acting (e.g. cortisol and inflammatory cytokines) and also those biomolecules that are thought to originate from and play a role in the central nervous system (e.g. GABA, NPY). Multiple reports evaluating PTSD and related outcomes suggest that circulating biomarkers that predict APNS might also reflect neurobiological changes occurring centrally[91–95]. For example, one study found a substantial overlap between biomarkers identified in post-mortem brain tissue and previously identified blood biomarkers of schizophrenia[92]. Another study found that gene expression in the blood and brain are comparable[96], suggesting that peripheral transcriptional programs can be representative of those in the central nervous system. If it is thought that peripherally detected biomarkers reflecting brain changes are particularly important to risk biomarker discovery for APNS, it might also be useful for researchers in the APNS field to consider examining neuronally derived microvesicles that transport specific biomolecules (e.g. miRNA, siRNA, mRNA) from the brain to peripheral tissues[97, 98]. These neuronal microvesicles are thought to communicate pathologic brain processes.

Key methodological considerations

Characteristics used to evaluate the potential utility of an APNS risk biomarker are summarized in Box 1.[99] In Figure 2 we outline key steps in the discovery pipeline of circulating blood-based risk biomarkers of APNS. Careful methodologic design is critical, because small differences in collection, isolation, and quantitation can greatly influence validity and translation. While myriad methodologic decisions must be made for every study, a selection of examples from various stages of the biomarker evaluation process will be presented here, using the evaluation of microRNA (miRNA) as an example.

- *Cohort and participant characteristics:* Perhaps one of the most critical factors influencing biomarker discovery is the quality of the epidemiological study in which biological samples are collected. This is because effect estimates of risk biomarker associations with APNS outcomes can be influenced by both selection biases, e.g. loss to follow-up[100], missing data[101], sampling bias[102] and information biases, e.g. due to interviewer[103], recall[104], or reporting[105] bias. Confounding[106] due to various participant characteristics and participant-centered factors (Figure 2) can also influence effect estimates.

- *Biofluid evaluated*: Biofluid selection must balance important trade-offs. For example, the use of serum or plasma (vs. whole blood) when searching for miRNA biomarkers has the advantages of enriching for non-blood based miRNA (such as neuronal-specific miRNA), and miRNA are stable in serum or plasma. However, the majority of miRNA in serum and plasma originate from blood cells (e.g. miR-451, miR-486) and inconsistent processing of serum and plasma can lead to inconsistent levels of these miRNA to be released into the extracellular compartment[107].

- *Timing of processing*: Time variation prior to processing (the amount of time that blood sits at room temperature following blood draw) can lead to inconsistent miRNA levels across samples due to ex-vivo transcription, degradation, and red blood cell lysis[108]. (Of note, plasma proteins are also susceptible to degradation, but might be stable for slightly longer periods of time than plasma based nucleic acids[109, 110])

- *Blood collection tube type:* Tube type (e.g., type and amount of anticoagulant) can influence miRNA quantification[111].

- *Sample storage*: Sample storage conditions, including storage temperature, number of freeze-thaw cycles, buffer used for storage, and length of time in storage can influence biomarker detection and quantification.[112]

- *Isolation method:* Methods of isolation and detection of the molecular component can influence biomarker detection. For example, miRNA can be isolated from plasma, serum, or whole blood using a variety of methods including both column-based (e.g. RNeasy kit) and column-free (e.g. Trizol) techniques. These techniques can affect downstream analyses and quantification, [113] and potential introduction of RNases at this step can degrade isolated miRNA.

- *Detection method*: Different biomarker detection methods have different technical biases that can create cross-method variation.[114–118] For example, different miRNA library preparation protocols for sequencing have been shown to generate discrepant results[119–121]. Fortunately, substantial efforts are being made to reduce technical biases[122, 123]. Additionally, due to the predominance of only a few highly abundant circulating biomolecules (e.g. the 22 most abundant circulating proteins account for 99% of the total protein concentration[124], and a small handful of miRNAs make up the vast majority of detectable miRNA), the

dynamic range of detection is greatly affected by the choice of processing platform. Therefore, detection methods (e.g. targeted arrays vs sequencing or mass-spectrometry) can influence the sensitivity of detection.

Following successful isolation and quantitation of biomarker data, it is important to perform proper quality control steps, such as examining the full cohort for outliers, technical errors, and batch effects. Quality controlled data is usually then normalized (e.g., for small RNA-seq reads, data normalization is standard, however, the normalization methods are not[125]) and in cases where the data is non-normally distributed, the data is often log transformed. Statistical and/or bioinformatics-based analyses are then applied to assess the relationship between candidate biomarkers and longitudinal APNS outcomes (e.g.,[126–128]). Important considerations include whether to take a hypothesis-driven vs. a data-driven approach, what factors to adjust for, and whether to perform traditional or machine-learning approaches.

Researchers should also consider whether they wish to develop a "stand-alone" biomarker or a biomarker that is applied in combination with clinical or other factors. Three examples of "stand-alone biomarkers are the FDA-approved RNA expression based tool, PAM50, that assesses risk of breast cancer recurrence[129], the microRNA expression based tool, Osteomir, that determines risk for a first fracture in female patients of postmenopausal osteoporosis[130–132], and the recent FDA-approved biomarker, Banyan-Brain Trauma Indicator (BTI), which assesses serum levels of two proteins GFAP and UCH-L1, to identify acute intracranial injury[133]. Perhaps the most well-know combined clinical and blood-based biomarker risk tools are heart disease vulnerability assessments, (e.g.,[134, 135]) which incorporate epidemiological factors (e.g., age, smoking status) and circulating blood-based biomarkers (e.g., HDL cholesterol, high sensitivity c-reactive protein[136]). Another approach is to use blood-based biomarkers in a tiered fashion, in which more expensive blood-based biomarkers are only used for individuals in whom epidemiologic and clinical factors alone do not achieve effective discrimination. An example of such a tiered use of biomarkers is current emergency department practice for the evaluation of pulmonary embolism among patients felt to be at low pre-test probability. Among such individuals, a tool using clinical/epidemiologic factors (The PERC rule[137, 138]) is first applied, and blood testing is only used among individuals in whom pulmonary embolism cannot be reasonably excluded using the PERC rule alone.

Translation to the clinic

Once a promising circulating risk biomarker or multi-biomarker signature is identified, it is critical that further development and testing be performed in ways that allow potential translation to clinical practice (see Box 1 and Figure 2 for considerations on early-stage discovery). The Critical Path Initiative is an FDA-led national strategy that aims to improve the way products are developed, tested, and manufactured[139]. This initative includes the Biomarker Qualification Program (BQP), a part of the FDA's Center for Drug Evaluation and Research that creates a pathway for translating biomarkers to clinical use by working with researchers or requestors to guide biomarker development. The goal of the BQP is to ensure that the biomarker is: a) suited to a well-defined COU, b) measured reliably and feasibly, and c) adequately performing to support the COU.

One of the first examples of a blood-based risk biomarker that has been accepted into the BQP are pancreatic islet cell autoantibodies as susceptibility/risk biomarkers of future development of Type 1 Diabetes.[140] While not all biomarkers need to be qualified, it is highly recommended that researchers performing studies that seek to develop risk biomarkers of APNS become familiar with this program. This is because qualifying a biomarker for a specific context of use (COU) improves the value of that biomarker by providing FDA documentation and guidance for use, enables the biomarker to be used in drug development programs specific to the COU, and provides evidence to support its use in clinical trials that identify new therapeutics. Further information can be found at: Biomarker Qualification Program.

Challenges to the discovery of circulating risk biomarkers of APNS

Research efforts that aim to identify susceptibility/risk biomarkers for APNS face several challenges and limitations. First, APNS are vastly heterogeneous diagnostic entities; for example, the categorical diagnosis of PTSD based on DSM-5 has been calculated to comprise 636,120 possible symptom combinations, each meeting diagnostic criteria for the disorder.[141] These different symptom profiles may also have differences in their potential etiologies and biomarker profiles, thus examining PTSD and other APNS as single diagnostic categories may severely limit our ability to discover reliable biomarkers. However, as the field moves towards a more succinct definition of APNS outcomes that better characterize the full symptom profiles of trauma survivors (versus traditional syndromic classifications studied in isolation)[4], we will increase our ability to identify accurate target groups to which blood-based risk biomarkers can more specifically identify vulnerable individuals.

Second, few large-scale longitudinal studies have been performed that track APNS in samples large enough to permit powerful and replicable findings. The importance of large sample sizes when examining neuropsychiatric endpoints is highlighted, for example, by genome-wide association studies, wherein the number of identified hits steadily increases with sample size.[142–144] However, small high-quality longitudinal cohort studies also have utility to biomarker researchers, as these cohorts can be used to identify risk biomarkers with large predicted effect sizes or to replicate findings from larger cohorts. In the event that a researcher does not have a sample size sufficient for omics based analyses, *in silico* modeling approaches (e.g.[145, 146]) can also help identify promising candidate biomarkers and thus reduce the number of hypotheses being tested in any single study.

Third, with the exception of studies listed in Table 1, most studies to date are cross-sectional in nature. Cross-sectional studies have limited ability to distinguish between risk/predictive vs. diagnostic biomarkers, to identify temporal changes in blood-based biomarkers after trauma, or to identify outcome trajectories in which risk biomarkers could accurately identify the long-term course of APNS symptoms following trauma exposure.

Fourth, previous studies have largely focused on single biological measures that are known to be influenced by a great many factors in addition to stress exposure. An example is cortisol, a commonly used marker that can be greatly influenced by factors such circadian

and ultradian rhythmicity, age, and sex.[147, 148] One area of current research is evaluating whether increased accuracy can be achieved by combining cortisol or other specific biological measures with additional biological factors or with sociodemographic factors (e.g.,[149]).

Finally, as described above, methodological/technical hurdles are present at each step of biomarker assessment, which threaten identification and/or replication. These myriad hurdles underscore the need for both technical and biological validation of findings.

The promise of the AURORA study to identify circulating risk biomarkers of APNS

The AURORA (Advancing Understanding of RecOvery afteR traumA) study is a publicly funded large-scale (n = 5,000 target cohort) Emergency Department-based prospective study of APNS development following trauma exposure. Study participants are enrolled in the early aftermath of trauma exposure and undergo multi-layered, cross-disciplinary assessments over the course of one year. These assessments include neurocognitive, self-report, physiological, digital phenotype, psychophysiological, neuroimaging, and genomic assessments. Using this data, AURORA investigators and other individuals in the research community can improve outcome phenotypes, develop prediction tools, and improve understanding of molecular mechanisms driving APNS. For a more detailed description of the full study, please refer to the AURORA methods paper[4].

Towards the goal of identifying circulating risk biomarkers, biological specimens (RNA PAXgene, and EDTA-plasma) are collected in the Emergency Department from all AURORA participants and at two weeks and six months from a subset of study participants that also complete neuroimaging and psychophysical assessments (target n=800 participants at each timepoint) (Figure 3). Blood specimens are also collected at the six month timepoint from a further subset of study participants (target n=2,200 individuals). Other biological specimens including DNA PAXgene tubes (for genotyping and methylation analyses) are also collected throughout the study.

All specimens are processed immediately after collection and stored at −80°C until batch shipment to the National Institute of Mental Health Repository and Genomics Resources (NIMH RGR) for long-term storage. Assessments of specimen quality occurs continuously throughout the study to ensure standardization (e.g., plasma processing procedures, timing of blood draw and freezing, adequate blood volume). Importantly, these samples (n=88,000 total tubes including each 250μl plasma aliquot) and all AURORA data will be available to the broader scientific community after Fall 2022.

Biological specimens for the AURORA study are collected in the very early aftermath of trauma exposure (usually within 24 hours), and APNS outcome measures are assessed over the course of a year. Therefore, circulating biomarker levels can be incorporated into traditional statistical models and contemporary machine learning algorithms that assess the predictive accuracy of biological molecules, ratios or signatures in isolation or concomitantly with additional AURORA study data from other research domains in

identifying risk of APNS trajectories and multidimensional outcomes. Because biological specimens are also collected in many participants at multiple timepoints, how circulating biomarkers change over APNS developmental or recovery trajectories can also be assessed.

Conclusions

In this literature review, we identified twenty-three studies that assessed circulating risk biomarkers of APNS. Eighty three percent of the identified studies were published in the past ten years. This recent increase in attention to the identification of APNS risk biomarkers, along with multiple recent efforts aimed at increased biomarker discovery (e.g. the FDA BQP, PTSD Biomarker Database, and AURORA Study), marks an exciting time for research in this field. This increased trajectory, together with continued methodologic advances, provide hope that the next decade will see substantial advances towards the goal of objectively identifying at-risk individuals and gaining insight into pathophysiologic mechanisms. However, these years will not be without immense challenges, especially those challenges related to methodological complexities of biomarker collection, processing, and analysis, integration of diverse efforts across laboratories and studies, replication across cohorts, and translation of our discoveries from the bench to bedside. While these research efforts are challenging, with a collaborative and thoughtful research process the future for circulating risk biomarker discovery for APNS is bright.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements and Disclosures:

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Box 1.

Ten characteristics of an ideal circulating blood-based susceptibility/risk biomarker of APNS, adapted from the FDA Biomarker Qualification Program and previously published literature, as indicated.

Biofluid collection is feasible.

Collection of biofluids should be feasible in care settings where individuals report following trauma (e.g. the emergency department), should be quick to collect, and collection should be minimally invasive. Whole blood, serum, and plasma are examples of such biofluids.

Biomarker measurement is feasible.

There should be one or more publicly accessible assays for measuring the biomarker, with potential for translation to clinically used, FDA approved assays/platforms, e.g. ELISA for protein quantification or Nanostring nCounter[1] for RNA expression measurements.

Biomarker measurement is reliable.

Test-retest reliability and reproducibility should meet assay analytical performance requirements acceptable to the FDA.

Context of use (COU) is clearly defined.

The context of use is a concise description of the biomarker's specified use. For example, a susceptibility/risk biomarker for APNS development would need to be indexed to a specific population (e.g. age of individuals, sex, ethnicity), a specific outcome (e.g. does the biomarker assess risk for PTS, depression, and CMP, just one of those outcomes, etc), and a specific timeframe of effectiveness (e.g. is it only useful if measured within the first 24 hours of trauma exposure, or can it be measured outside of that timeframe). A more thorough guideline for defining COU can be found here: FDA COU

Biomarker assesses risk of APNS development both sensitively and specifically.

These performance measures, along with positive predictive values and negative predictive values should be carefully measured and considered in the context of clinical utility[15, 16]. For example, for APNS risk assessment it might be more acceptable to have a biomarker with more false positives than false negatives if the treatment regimen is low risk (e.g. cognitive behavioral therapy for PTS), whereas it might be more acceptable to have a biomarker with increased false negatives if the treatment regimen is high risk (e.g. long-term opioid therapy for CMP) or is expensive.

Biomarker consistently identifies at-risk individuals across ethnicities and sexes.

Recent evidence indicates that longstanding risk tools such as those predicting cardiovascular disease are limited in their ability to assess risk across varied ethnicities[21]. Therefore, biomarker discovery should be performed independent of these factors or should specifically stratify on these factors and clarify this limitation in the COU.

Analytical performance adequately supports COU.

Measurement of a biomarker's technical performance and clinical utility should support the defined COU.

Biomarker test results can be generated rapidly.

Rapid assays facilitate the ability to risk stratify early after trauma exposure such that interventions can be administered when potentially most efficacious[6, 13, 14, 22–27]. Rapid test results also improve patient satisfaction.

Measurement of the biomarker is inexpensive.

Concerted efforts should be made to prioritize low-cost biomarkers over high-cost biomarkers in order to improve access to care for all individuals.

Biomarker discovery studies should translate between human and animal.

Parallel evidence in humans and in appropriate models of APNS is important in order to further elucidate details about the biomarker that might not be feasible in human studies. For example, animal studies might enable granular resolution of the timing of increased/decreased levels of the biomarker, extrapolation of results to gain insights into neurobiology of nervous system tissue, and in assessing the utility of the biomarker in other settings such as in drug discovery efforts to identify novel therapeutics that prevent APNS.
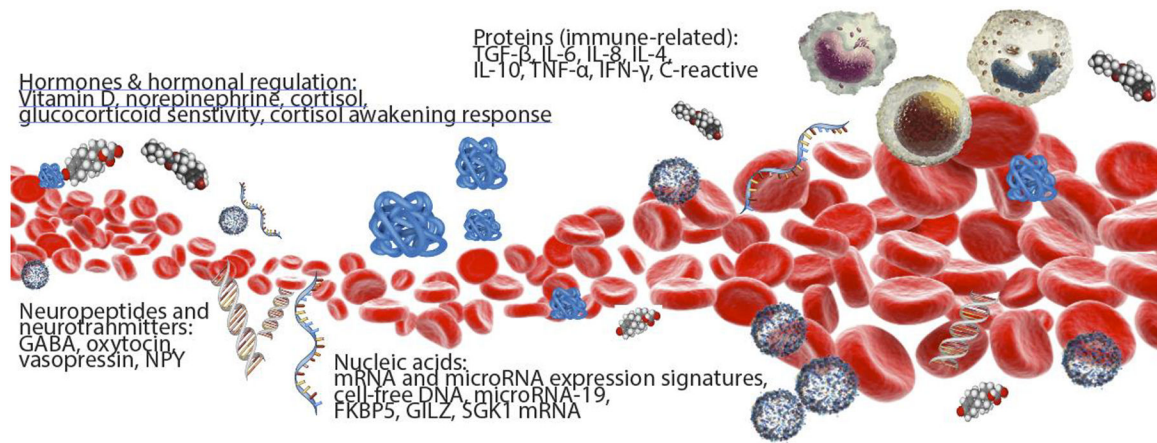
Figure 1.
Circulating blood-based risk biomarkers that have been assessed in previous studies for their ability to predict the development of adverse posttraumatic neuropsychiatric sequelae (APNS). General categories for these risk biomarkers include hormones, proteins, immune mediators, nucleic acids, and neuropeptides and neurotransmitters. Note that not all circulating risk biomarkers that have been examined to date showed a statistically significantly relationship with APNS. See Table 1 for more details. Validation of the significantly associated risk biomarkers and identification of novel circulating risk biomarkers within the described categories or outside of these categories (e.g. metabolites, microvesicles, immune cells) will be a high priority for APNS-focused researchers in the coming years.
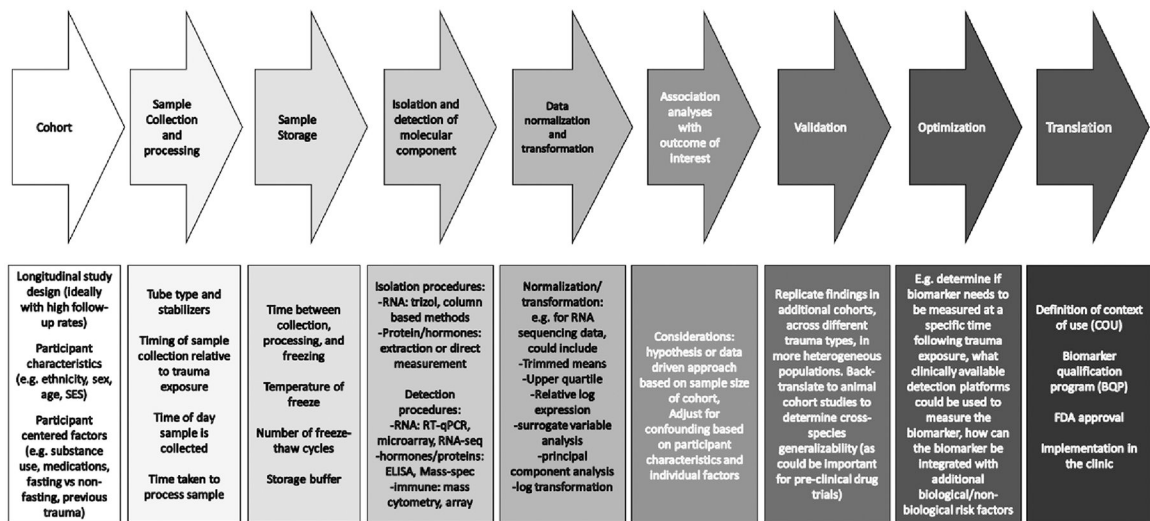
Figure 2.

Key steps (top) and methodological considerations (bottom) in the discovery of circulating blood-based risk biomarkers of adverse posttraumatic neuropsychiatric sequelae (APNS). While the steps and considerations included in this figure are not exhaustive, it represents a subset of the myriad factors that can influence blood based biomarker discovery and translation. For instance, the epidemiological design of the cohort from which samples are drawn, and characteristics/factors of participants can determine whether there is sufficient statistical power to detect a risk biomarker, whether adjustors should be included in statistical models and whether the findings are generalizable to additional populations. SES = socioeconomic status, FDA = U.S. Food and Drug Administration. Adapted from a variety of sources including:[99, 119, 150]
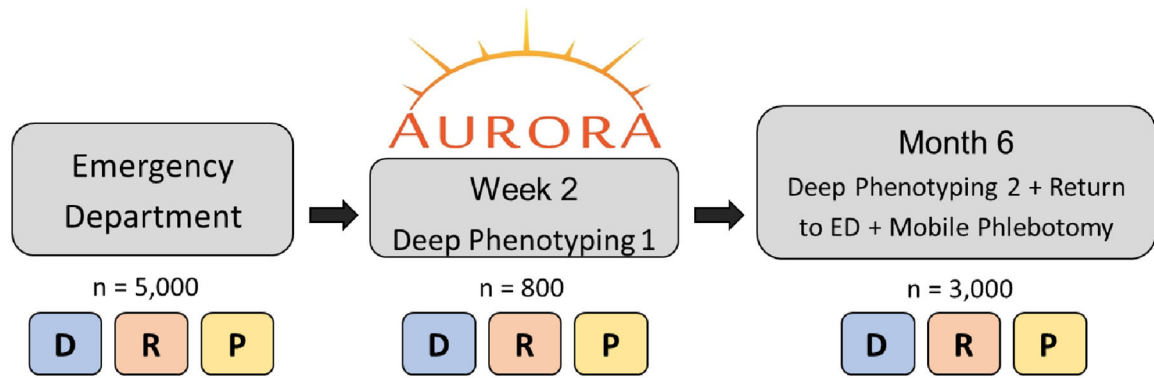
Figure 3.
The AURORA study is an on-going longitudinal cohort study assessing APNS development following trauma exposure. Individuals are enrolled in the Emergency Department and followed over the course of a year. Consistent with the theme of this review article, one main goal of the AURORA study is to discover circulating risk biomarkers that identify vulnerable individuals in the early aftermath of trauma exposure. Therefore, blood samples are collected from all individuals immediately following enrollment (n=5,000; left). Additional blood samples are collected from a subset of participants two weeks (n=800; middle) and six months (n=3,000; right) following trauma exposure. These longitudinal samples are collected at either Deep Phenotyping sessions when additional multilayered data are collected (e.g. functional and structural MRI, pain physiology, startle response) or via Mobile phlebotomy/participant return to the Emergency Department. Longitudinal blood samples can be used to assess trajectories of risk biomarkers and/or to identify diagnostic biomarkers using nested case-control samples. D = DNA PAXgene tube, R = RNA PAXgene tube, P = EDTA tube for plasma, ED = Emergency Department. All sample sizes represent planned cohort sizes based on enrollment rates and funding as of the date of publication of this review. All samples and processed data derived from the AURORA cohort will be available to the full research community in Fall 2022.

Table 1.

Summary of results from previous longitudinal studies examining circulating blood-based susceptibility/risk biomarkers of adverse posttraumatic neuropsychiatric sequelae (APNS)

Reference	Type of APNS	Sample Size/ Description	Clinical measures	Biological markers	Main Finding
Vaiva et al. 2004[81]	PTSD	108 motor vehicle collision survivors presenting to ED	DSM IV criteria for PTSD given 6 weeks after trauma	GABA levels in plasma γ	Lower plasma GABA levels are associated with higher risk for PTSD development.
Segman et al. 2005[67]	PTSD	33 male and female trauma survivors presenting to the ED	DSM IV PTSD criteria given 1 and 4 months after trauma	Genome-wide mRNA expression in PBMC	Peripheral gene expression signatures following trauma identify evolving PTSD and are informative of its key clinical features and outcome.
Pervanidou et al. 2007[89]	PTSD	56 children and adolescents enrolled following motor vehicle collision; matched to 40 controls	PTSD part of the K-SADS-PL given 1 and 6 months after trauma	serum and salivary cortisol, serum IL-6 and plasma catecholamines	Increased peritraumatic circulating morning IL-6 levels and increased evening salivary cortisol levels predicted PTSD 6 months later
Cohen et al. 2011[78]	PTSD	48 patients hospitalized after orthopedic injuries and 13 gender-matched healthy volunteers	PDSS given 1 month after trauma exposure	Serum levels of multiple cytokines, including IL-6, IL-8, TGF-β, IL-4, and IL-10	Higher levels of IL-8 and lower levels of TGF-β were associated with subsequent higher PTSD symptoms.
Inslicht et al 2011[79]	PTSD	296 police officers enrolled during academy training	DSM IV criteria for PTSD given 12, 24, 36 months after training	Cortisol awakening resonse (change in cortisol from first awakening to 30 minutes later)	Pre-trauma cortisol awakening responses did not predict PTSD symptoms.
Van Zuiden et al. 2011[68]	PTSD	68 male Dutch military personnel deployed to Afghanistan	SRIP given 6 months after deployment	GR Number and mRNA expression of GR targets in PBMC	Predeployment higher GR number, but not mRNA expression of GR targets, predicts risk for the development of PTSD symptoms after military deployment.
Van Zuiden et al. 2012[69]	PTSD	448 male Dutch military personnel deployed to combat	SRIP given 6 months after deployment	GR Number and mRNA expression of GR targets in PBMC	Predeployment higher GR number, lower *FKBP5* mRNA, and higher *GILZ*, but not *SGK1* mRNA or cortisol levels, predict PTSD after military deployment.
Glatt et al. 2013[70]	PTSD	48 male US marines deployed to Iraq or Afghanistan	PCL score	Genome-wide mRNA expression in leukocytes	Dysregulated gene expression profiles enriched for immunity-related genes precede the development of PTSD.
Eraly et al. 2014[80]	PTSD	2208 male infantry battalions imminently deploying to a war zone	CAPS given 3 and 6 months after deployment	Plasma CRP levels	Plasma CRP was prospectively associated with PTSD symptoms.
Van Zuiden et al. 2015[71]	PTSD	721 male and female Dutch soldiers deployed to Afghanistan	SRIP and SCL-90 given 1 and 6 months after deployment	Glucocorticoid sensitivity in whole blood	Pre-deployment glucocorticoid sensitivity predicts PTSD and depression symptoms 6 months after deployment.
Breen et al. 2015[88]	PTSD	94 male U.S. marines (dataset I) and 48 male U.S. marines (dataset II) deployed for combat in Iraq or Afghanistan;	CAPS given 1 month pre and 3 months post deployment	Leukocyte mRNA expression using co-regulated gene networks	Over-expression of genes enriched for functions of innate-immune response and interferon signalling (Type-I and Type-II) as resiliency signatures.
Gandubert et al. 2016[90]	PTSD	123 male and female individuals enrolled in	French version of the Watson's PTSD	cortisol, norepinephrine,	higher levels of 12 h-overnight urinary norepinephrine

Reference	Type of APNS	Sample Size/ Description	Clinical measures	Biological markers	Main Finding
		the ED 2–7 days following criterion A1 or A2 trauma exposure	Interview given 1, 4, and 12 months post-trauma	epinephrine, CRP, total and HDL cholesterol, glycosylated haemoglobin	predicted PTSD at 4 months following trauma exposure
Reijnen et al. 2017[72]	PTSD	907 male Dutch military personnel deployed to Afghanistan	SRIP given 1 and 6 months and 1, 2, and 5 years after deployment	Plasma oxytocin and vasopressin levels	Pre-deployment oxytocin and vasopressin levels did not significantly predict PTSD symptoms up to 5 years after deployment.
Reijnen et al. 2018[77]	PTSD	3319 Dutch male military personnel deployed to Iraq or Afghanistan	SRIP and CAPS up to 6 months after deployment	Plasma neuropeptide Y	Predeployment plasma NPY was not associated with PTSD symptoms over time.
Michopoulos et al. 2019[44]	PTSD	274 participants presenting to the ED after trauma exposure	PSS delivered 1, 3, 6, and 12 months after trauma	Plasma levels of twenty-seven cytokines, chemokines, and growth factors	Lower TNFα and IFNγ levels at the time of ER presentation were associated with chronic PTSD. None of the other measured markers were associated with PTSD outcomes.
Vaiva et al. 2006[76]	PTSD Depression	78 motor vehicle collision survivors presenting to ED	DSM IV criteria for PTSD and major depression 6 weeks and 1 year after trauma	GABA levels in plasma	A plasma GABA level below 0.20 mmol/ml is associated with chronic PTSD and depression.
Van Zuiden et al. 2012[74]	PTSD, Depression	526 male Dutch military personnel deployed to Afghanistan	SRIP and SCL-90 given 6 months after deployment	Glucocorticoid sensitivity in whole blood; GR number and mRNA expression of gene targets in PBMC	Lower glucocorticoid sensitivity predicts lower PTSD and higher depression symptoms 6 months after military deployment. GR pathway components predict 6-month PTSD symptoms only.
Walsh et al. 2013[73]	PTSD, Depression	235 female sexual assault survivors presenting to the ED	PSS-SR and BDI given 6, 12, and 24 weeks after trauma	Serum cortisol	Higher ER cortisol levels predict higher PTSD and depression symptoms 6 weeks after trauma but lower symptoms over time.
Yu et al. 2018[75]	PTSD, Pain	65 African American female motor vehicle collision survivors	Impact of Event Scale and modified regional pain scale given 6 months after trauma	mRNA expression of X chromosome gene transcripts in whole blood	Genes known to escape X chromosome inactivation predict co-morbid chronic musculoskeletal pain and posttraumatic stress symptom development in women following trauma exposure.
Linnstaedt et al. 2019[84]	PTSD, Pain	179 African Americal male and female motor vehicle collision survivors presenting to the ED and 74 female sexual assault survivors presenting to SANE sites	Impact of Event Scale and modified regional pain scale given 6 months after trauma	MicroRNA-19b expression in whole blood	microRNA-19b predicts risk for PTSD and chronic pain in a sex-dependent manner following trauma. Relationship between microRNA-19b and PTSD in women was replicated across two independent cohorts.
Linnstaedt et al. 2015[82]	Pain	53 African American male and female motor vehicle collision survivors presenting to the ED	Numeric rating (0 −10) pain scale given 6 weeks after trauma	MicroRNA expression in whole blood	MicroRNAs circulating in the early aftermath of motor vehicle collision predict persistent pain development in sex-specific and suggest a role for microRNA in pain differences.
Mauck et al. 2019[85]	Pain	133 African American male and female motor vehicle collision	Numeric rating (0 −10) pain scale given 6 weeks, 6	Plasma Vitamin D levels	Low Vitamin D levels in the peritraumatic period predict higher pain levels over the

Reference	Type of APNS	Sample Size/ Description	Clinical measures	Biological markers	Main Finding
		survivors presenting to the ED	months, and 1 year after trauma		course of a year following motor vehicle collision trauma
Rushton et al. 2018[83]	Pain, Disability	500 male and female patients with acute musculoskeletal trauma	Chronic Pain Grade Scale given 6 months after trauma	CRP and cfDNA in plasma	Protocol for developing a screening tool to predict chronic pain and disability after musculoskeletal trauma.

Abbreviations: BDI, Beck Depression Inventory; cfDNA, cell-free DNA; CRP, C-reactive protein; DSM, diagnostic and statistical manual for mental disorders; ED, emergency department; SANE, sexual assault nurse examiner; GR, glucocorticoid receptor; PBMC, peripheral blood mononuclear cells; PCL, PTSD Checklist based on the Clinician-Administered PTSD Scale (CAPS); PDSS, Posttraumatic Disorder Symptom Scale (PDSS); K-SADS-PL, Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime version; PSS-SR, PTSD Symptom Scale—Self-Report; PTSD, posttraumatic stress disorder; SCL-90, 16-item Symptom Checklist–90 depression subscale; SRIP, Self-rating inventory for PTSD; VAS, 10cm visual analogue scale (0–10) for assessing pain.