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Review

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Trauma diagnostic-related target proteins and their detection techniques

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Abstract

Trauma is a significant health issue that not only leads to immediate death in many cases but also causes severe complications, such as sepsis, thrombosis, haemorrhage, acute respiratory distress syndrome and traumatic brain injury, among trauma patients. Target protein identification technology is a vital technique in the field of biomedical research, enabling the study of biomolecular interactions, drug discovery and disease treatment. It plays a crucial role in identifying key protein targets associated with specific diseases or biological processes, facilitating further research, drug design and the development of treatment strategies. The application of target protein technology in biomarker detection enables the timely identification of newly emerging infections and complications in trauma patients, facilitating expeditious medical interventions and leading to reduced post-trauma mortality rates and improved patient prognoses. This review provides an overview of the current applications of target protein identification technology in trauma-related complications and provides a brief overview of the current target protein identification technology, with the aim of reducing post-trauma mortality, improving diagnostic efficiency and prognostic outcomes for patients.

Introduction

Trauma is a major health issue. According to estimates from the Global Burden of Disease Study, trauma is responsible for around 10% of global deaths (Ref. 1). Significant progress has been made in managing trauma patients because of the continuous advancement of medical technology. Rational and effective treatment and care protocols have significantly improved the survival rates of trauma patients (Ref. 2). Nonetheless, trauma patients still face a significant threat to their survival because of a range of complications. Multiple studies have reported potential complications that trauma patients may encounter, as shown in Figure 1, including psychological trauma-related disorders (Ref. 3), as well as various physiological complications such as infection-induced sepsis (Refs 4, 5), thrombosis or haemorrhage (Refs 6, 7), ischaemic tissue necrosis (Ref. 8), acute respiratory distress syndrome (Ref. 9), traumatic brain injury (TBI) (Ref. 10), among others. Trauma patients who are admitted to the intensive care unit (ICU) often face an increased risk of complications, which contributes to a substantial mortality rate. Statistics indicate that among trauma patients who die several days or weeks after the incident, 45% of deaths can be attributed to severe brain injury, adult respiratory distress syndrome (ARDS) or multiple organ dysfunction syndrome (Ref. 11).

Timely detection of newly emerging infections and complications in trauma patients is crucial as it enables physicians to initiate timely and appropriate treatment measures, thereby reducing mortality rates and improving patient prognosis after trauma. Severe trauma can worsen systemic inflammation in patients, often leading to sepsis and life-threatening consequences. Traditionally, monitoring severely injured trauma patients has primarily relied on assessing the extent of injury. However, by employing target protein identification of characteristic biomarkers in trauma, precise assessment, monitoring and prediction of trauma outcomes can be achieved (Ref. 12). Therefore, target protein identification techniques can be vital tools in clinical diagnostics, particularly for early identification of biomarkers for various trauma complications. Additionally, they can offer comprehensive protein information to explore the pathogenesis and progression of related diseases, uncover intricate cellular metabolic pathways and signalling networks and identify key factors and associated proteins involved in disease development. This enhances our understanding of diseases and facilitates further research, drug design and the development of treatment strategies (Refs 13, 14). In accordance with the illustration provided in Figure 1, some of the target protein identification techniques currently being developed include mass spectrometry (MS), polyacrylamide gel electrophoresis (PAGE) and immunological methods. By selectively utilizing these techniques based on specific needs, it is possible to identify and validate target proteins associated with the

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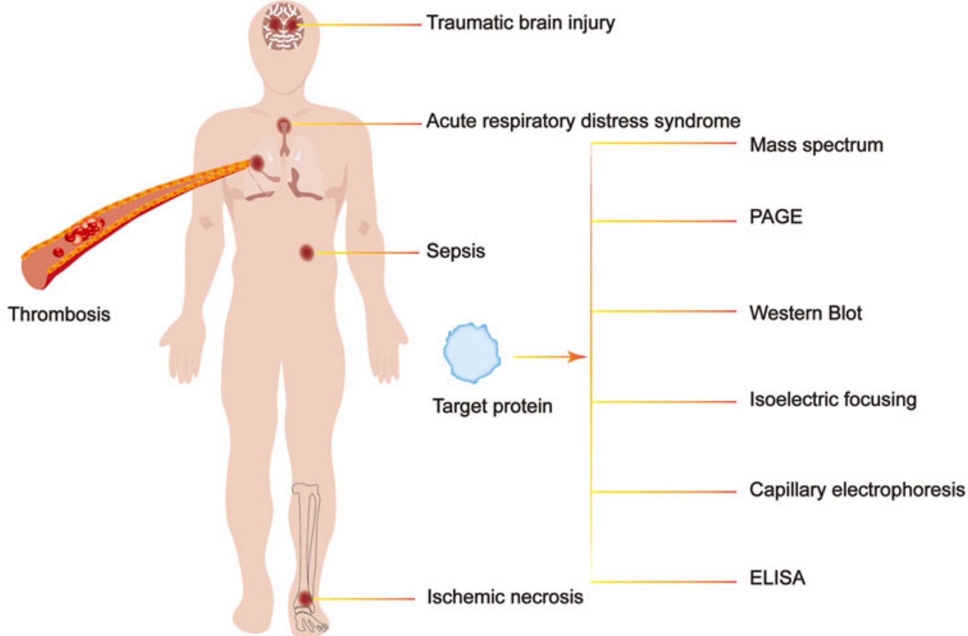

Figure 1. Classification of complications of trauma and methods for identifying their target proteins.

occurrence or prognosis of trauma-related complications. This enables timely diagnosis and improves the post-trauma survival rate of patients. In order to contribute to the reduction of post-trauma mortality rates and enhance diagnostic efficiency and prognostic outcomes for patients, the aim of this review is to comprehensively gather and evaluate promising biomarkers along with the methods employed for their detection. By consolidating and analysing this information, it can be applied to effectively improve the survival rate post-trauma. In the following sections, we will explore the various consequences of trauma and their associated biomarkers, where we will delve into the biomarkers that play a crucial role in its diagnosis and prognosis. Afterwards, we will also review the detection methods related to complications following trauma.

Post-traumatic sepsis

In 1991, the American College of Chest Physicians and the Society of Critical Care Medicine jointly released the first version of international consensus defining sepsis during the consensus conference. According to the conference, sepsis is described as a progressive continuum of inflammatory deterioration, starting from systemic inflammatory response syndrome triggered by infection and advancing to sepsis, severe sepsis and septic shock. Nevertheless, this definition had notable limitations as it failed to consider specific biomarkers that commonly elevate during sepsis (Ref. 15). The third edition of the international consensus definition for sepsis and septic shock introduced a more precise definition and advocated the use of the quick sequential organ failure assessment as an efficient screening tool for sepsis to identify patients with suspected infections and unfavourable prognoses (Ref. 16). Sepsis can result in diverse organ damages, encompassing sepsis-associated encephalopathy, septic cardiomyopathy, sepsis-associated acute kidney injury, sepsis-associated acute lung injury and sepsis-induced liver injury (Ref. 17). Without a doubt, early identification of the infection source and initiation of antibiotic therapy in a timely manner are vital measures to decrease mortality rates in sepsis, septic shock and trauma patients. Timely identification of the infection source enables healthcare professionals to expeditiously implement suitable treatment measures, including initiating appropriate antibiotic therapy, effectively controlling infection spread and preventing the emergence of severe complications.

Presently, extensive research has demonstrated the significant diagnostic, therapeutic and prognostic roles of sepsis biomarkers, which exhibit a direct correlation with the severity of the injury (Ref. 18). Biomarkers associated with sepsis include cytokines, tumour necrosis factor-alpha (TNF-α), markers of cellular pyroptosis, high-density lipoprotein (HDL), procalcitonin (PCT) and C-reactive protein (CRP), among others. Furthermore, each of these biomarkers will be sequentially introduced in the subsequent sections. And the summarized information is provided in Table 1.

Cytokines

Cytokines, which are small proteins secreted by both immune and non-immune cells, exhibit diverse biological activities and play a pivotal role in immune response (Ref. 19). Studies have demonstrated the diagnostic potential of interleukin-6 (IL-6) for early-onset neonatal sepsis (Ref. 20). IL-6 is a multifunctional cytokine involved in immune response, tissue regeneration and metabolism. Rapid production of IL-6 contributes to host defence during infection and tissue damage, whereas excessive synthesis of IL-6 and dysregulation of IL-6 receptor signalling are associated with pathological conditions (Ref. 21). Thus, IL-6 serves as a reliable biomarker for inflammatory diseases. Elevated serum IL-6 levels not only indicate sepsis in patients, but also reflect the severity of sepsis with significant and rapid changes (Ref. 22). Moreover, research conducted by Chen *et al.* suggests that the elevation of IL-6 levels worsens sepsis and leads to myocardial injury (Ref. 23).

Tumour necrosis factor-alpha

TNF-α is a polypeptide polymer that exhibits diverse biological effects. TNF-α can activate T cells and stimulate the production of pro-inflammatory mediators such as IL-1 and IL-6, ultimately leading to the development of a cytokine storm (Ref. 24). A study on the influence of cytokine levels on immune status and survival

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Table 1. Biomarkers of post-traumatic sepsis and their biological functions

Biomarkers	Biological functions
IL-6	Plays a pivotal role in immune response, tissue regeneration and metabolism
TNF-α	Activates T cells and stimulates the production of pro-inflammatory mediators such as IL-1 and IL-6
Inflammatory enzyme caspase-1	Mediates cell pyroptosis and triggers a systemic inflammatory response in the host
HDL	Demonstrates protective functions against atherosclerosis
PCT	Under pathological conditions such as inflammation and infection, it undergoes further processing to form calcitonin
CRP	Facilitates the binding of damaged and disrupted cells and contributes to the clearance of debris generated from tissue damage
PTX3	Secreted in response to inflammatory stimuli and regulates immune responses and inflammatory processes

outcomes in elderly patients with sepsis indicated that, it was found that TNF-α levels in the serum of patients in both the mild and severe sepsis groups were significantly higher than those in the control group ($P < 0.05$). Additionally, TNF-α levels were significantly higher in the severe sepsis group compared with the mild sepsis group ($P < 0.05$) (Ref. 25). This suggests a direct correlation between TNF-α levels and the condition as well as the prognosis of sepsis patients.

Markers of cellular pyroptosis

Cell pyroptosis is a form of programmed cell death that is triggered by pro-inflammatory mediators (Ref. 26). According to the study by Wang *et al.*, trauma patients who develop sepsis frequently exhibit a higher proportion of peripheral blood mononuclear cells (PBMCs) undergoing pyroptosis compared with those who do not develop sepsis (Ref. 27). During the early stages of trauma, there is a notable rise in the proportion of PBMCs undergoing pyroptosis, which exhibits a significant correlation with the severity and inflammatory status of trauma patients. Cell pyroptosis is primarily mediated by the inflammatory enzyme caspase-1 and triggers a systemic inflammatory response in the host. Therefore, elevated expression of caspase-1 frequently signifies the onset of sepsis, and PBMC pyroptosis emerges as a potential biomarker for predicting sepsis development following trauma.

High-density lipoprotein

HDL is a complex family of lipoprotein particles consisting of diverse biomolecules, such as proteins and lipids. HDL particles demonstrate protective functions against atherosclerosis in various cell types. These functions encompass promoting cholesterol efflux from macrophages within the arterial wall, exerting antioxidant activity, demonstrating anti-inflammatory effects and safeguarding the vascular endothelium (Ref. 28). According to the study by Tanaka *et al.*, lipid profiles of sepsis patients in the ICU significantly differ from those of general trauma patients. Sepsis patients exhibit lower levels of HDL, which possesses cardiovascular protective effects, whereas the HDL concentration in trauma patients remains unaltered (Ref. 29).

Procalcitonin and C-reactive protein

PCT and CRP have been identified in numerous studies as diagnostic and prognostic biomarkers for sepsis (Refs 30, 31, 32). PCT is a precursor protein synthesized by thyroid C cells (parafollicular cells). Under pathological conditions such as inflammation and infection, it undergoes further processing to form calcitonin. Therefore, it has been proposed as a specific biomarker for bacterial infection and has subsequently been applied in clinical practice (Ref. 33). CRP is a well-known acute-phase reactant, initially discovered in the 1980s and named for its capability to bind to pneumococcal C-polysaccharide. CRP plays a role in the apoptotic process by facilitating the binding of damaged and disrupted cells, and contributes to the clearance of debris generated from tissue damage (Ref. 34). Yang *et al.* suggested that detecting the expression levels of neutrophil CD64 and PCT can provide a more sensitive diagnosis of sepsis compared with CRP (Ref. 32).

In addition to diagnosing and predicting sepsis through biomarkers, Daigo *et al.* employed effective immunopurification methods to identify the proteomic characteristics of the sepsis pentraxin 3 (PTX3) complex and its interactions with neutrophil extracellular traps. PTX3 is a conserved glycoprotein (GP) that is secreted by various cells such as endothelial cells, myeloid cells, fibroblasts and osteogenic cells in response to inflammatory stimuli, and it plays a crucial role in regulating immune responses and inflammatory processes (Ref. 35). Their research suggests that PTX3 may help create an antimicrobial microenvironment in sepsis through its interaction with antimicrobial proteins (Ref. 36). Similar studies indicate that target protein identification techniques play a crucial role in enhancing our understanding of sepsis pathogenesis.

Trauma-related acute respiratory distress syndrome

ARDS is a severe inflammatory lung condition characterized by severe respiratory failure. It is characterized by persistent refractory hypoxaemia and the presence of diffuse opacities on chest radiographs (Ref. 37). A multinational prospective study conducted in 459 ICUs across 50 countries/regions on five continents revealed regional variations in the mortality rate of ARDS, but the overall mortality rate remains very high at approximately 40% (Ref. 38). Currently, treatment options for ARDS are limited to providing supportive measures aimed at preventing further lung damage. For patients who do not respond well to these measures, the mortality rate is even higher, reaching 60% (Ref. 39). ARDS, a severe lung condition, can be caused by various factors such as sepsis, trauma, smoking and blood transfusions. Its severity and complexity pose challenges in treatment and management (Refs 40, 41).

Many biomarkers have been tested for the diagnosis and treatment of ARDS (Ref. 42). However, ARDS induced by trauma exhibits clinical and biological differences compared with ARDS resulting from other clinical conditions (Ref. 43). Biomarkers for ARDS can be categorized as endothelial cell injury biomarkers (e.g. plasma angiopoietin-2 (Ang-2) (Ref. 44), von Willebrand factor (vWF) (Ref. 45), vascular endothelial growth factor (VEGF) (Ref. 46)), epithelial injury biomarkers (e.g. surfactant protein-D (SP-D)) (Ref. 47), soluble receptor for advanced glycation end

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products (sRAGE) (Ref. 48), Clara cell protein 16 (CC-16) (Ref. 49)), inflammatory cytokines (e.g. various interleukins (ILs) (Ref. 50), mitochondrial DNA (Ref. 51)) and other biomarkers (Ref. 42). Similarly, they are consolidated and summarized in Table 2.

Endothelial cell injury biomarkers

Ang-2, as an endothelial cell growth factor, plays a crucial role in regulating vascular permeability and has been identified as a key mediator in acute lung injury (ALI) in animal models (Ref. 44). As a biomarker, Ang-2 can distinguish trauma-related ARDS from general ALI (Ref. 50). Furthermore, studies have demonstrated that a higher ratio of Ang-2 to angiopoietin-1 (Ang-1), with Ang-1 acting as an antagonist of Ang-2, is an independent predictor of mortality in patients with lung injury (Ref. 52). vWF is a GP involved in haemostasis and is found in endothelial cells (Ref. 45). Research by Moss *et al.* suggests that vWF levels are associated with the aetiology of ARDS in patients, allowing for the identification of trauma-related ARDS based on vWF levels (Ref. 53). VEGF is a significant cytokine that promotes angiogenesis in the body (Ref. 54). Medford *et al.* demonstrated that genetic polymorphisms in the VEGF pathway are associated with an increased risk of developing ARDS in ventilated patients (Ref. 46).

Epithelial injury biomarkers

SP-D is a pulmonary surfactant protein primarily located in alveoli and respiratory epithelial cells. It plays a central role in regulating lung host defence and modulating allergic responses (Ref. 55). The receptor for advanced glycation end products (RAGE) and its soluble form (collectively known as sRAGE) are believed to be involved in host defence against infection, inflammation, cardiac metabolic disorders and age-related diseases (Ref. 56). Research by Uchida *et al.* demonstrated that RAGE is an effective biomarker for ARDS, and the levels of RAGE in the serum vary depending on the severity of lung injury (Ref. 48). CC-16 is a secretory protein primarily located in the terminal bronchiolar Clara cells along the bronchial tree. Increasing evidence suggests that CC-16 plays a crucial protective role against oxidative stress and inflammatory in the respiratory tract (Ref. 57). Research by Ware *et al.* confirmed that three biomarkers produced by lung epithelium (SP-D, RAGE and CC-16) can have good discriminatory power for the potential diagnosis of ARDS in septic patients (Ref. 47).

Inflammatory cytokines

ILs are a class of protein signalling molecules produced by immune system cells, and they exhibit diverse biological activities and regulatory functions in immune responses. Notably, plasma biomarkers such as IL-6 and IL-8 have been confirmed to be valuable in diagnosing traumatic lung injury (Ref. 50). Furthermore, Faust *et al.* demonstrated a significant association between plasma mitochondrial DNA levels and ARDS in trauma patients (Ref. 51).

Other biomarkers

Ang-2, CC-16 and mitochondrial DNA have been demonstrated to possess diagnostic or prognostic value in trauma-related ARDS (Refs 50, 58). However, there are still many biomarkers that have not been reported yet, which can aid in the diagnosis of ARDS. The development of genomics, metabolomics and proteomics has led to the identification of an increasing number of differentially expressed genes, metabolites and proteins specific to ARDS. Dong *et al.* utilized proteomic analysis to identify insulin-like growth factor-binding protein 7 (IGFBP7) as a novel pathogenic protein in ARDS. IGFBP7 is a protein that binds to insulin-like growth factors (IGFs), thereby modulating their activity and biological effects within cells. The team found that plasma IGFBP7 is associated with the 28-day mortality rate in ARDS, providing a foundation for further experimental and clinical research on the mechanisms and utility of IGFBP7 as a biomarker for this disease (Ref. 59). Additionally, Rademaker *et al.* proposed a targeted proteomics analysis method to investigate the thrombotic pathways in ARDS associated with COVID-19 and influenza. This study demonstrated the significance of the technique in elucidating the pathological mechanisms of ARDS (Ref. 60).

Traumatic brain injury

TBI is a brain injury caused by external mechanical forces, affecting millions of people worldwide. The causes of TBI are diverse and include sports-related injuries, road traffic accidents, falls, assaults and other factors (Ref. 61). Recently, falls have surpassed road traffic accidents as the primary cause of TBI (Ref. 62). The threat posed by TBI should not be underestimated. On the one hand, there is currently no cure for TBI, and drug development for this condition has encountered frequent failures in clinical trials. On the other, accurately determining injury phenotypes in cases of mild damage is a challenging task (Ref. 63). A study by Thornhill *et al.* showed that the majority (78%) of patients with severe TBI experienced disabilities. Similarly, the disability rate was 54% in moderate TBI patients and 51% in mild TBI patients (Ref. 64). Early diagnosis of TBI commonly involves a combination of imaging techniques and clinical studies, whereas biomarkers play a crucial role in identifying biological processes

Table 2. Biomarkers of trauma-related ARDS and their biological functions

Biomarkers	Biological functions
Ang-2	Regulates vascular permeability
vWF	GP involved in haemostasis found in endothelial cells
VEGF	Cytokine that promotes angiogenesis in the body
SP-D	Regulates lung host defence and modulates allergic responses
sRAGE	Involved in host defence against infection, inflammation, cardiac metabolic disorders and age-related diseases
CC-16	Plays a protective role against oxidative stress and inflammation in the respiratory tract
Various ILs	Diverse biological activities and regulatory functions in immune responses
Mitochondrial DNA	Has a significant relationship with ARDS in trauma patients
IGFBP7	Binds to and regulates IGFs

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that are not directly observable through imaging techniques. Biomarkers can provide information about ongoing tissue damage and the risk of neurological deterioration, making them valuable supplementary diagnostic tools. Therefore, diagnosing different severity levels of TBI and predicting and improving outcomes through biomarker identification hold significant value. The detection of novel biomarkers can offer insights into the underlying mechanisms of TBI, aid in early diagnosis, predict disease progression and contribute to the development of highly clinically significant molecular-targeted therapies.

Previous literature has reported several biomarkers associated with TBI. Typically, biomarkers for TBI are detected in blood or cerebrospinal fluid (CSF). Inflammatory cytokines such as TNF-α and interleukin-1 beta (Ref. 65), similar to sepsis and ARDS, may suggest the presence of TBI. However, they lack specificity as reliable biomarkers for TBI.

The most extensively studied biomarkers in TBI research are those related to astrocyte injury, primarily S100 calcium-binding protein B (S100B) (Refs 66, 67) and glial fibrillary acidic protein (GFAP) (Refs 68, 69). S100B is a calcium-binding protein secreted by astrocytes, Schwann cells, melanocytes, adipocytes and chondrocytes (Ref. 70). Haselmann *et al.* demonstrated that S100B is a reliable biomarker for TBI. They also found that plasma-based S100B detection is superior to serum-based detection, significantly reducing processing time (Ref. 71). GFAP is a specific intermediate filament protein found in astrocytes and is upregulated during astrocyte proliferation. Research by Vos *et al.* has shown that both GFAP and S100B can be used to assess the severity of brain injury following TBI. When combined with clinical variables and subjected to comprehensive analysis, these biomarkers may help in more accurately predicting patient prognosis (Ref. 69).

Besides the abovementioned biomarkers, there are other protein biomarkers associated with neuronal cell body injury, such as ubiquitin C-terminal hydrolase-L1 (UCH-L1) (Ref. 72), neuronal-specific enolase (NSE) (Ref. 73) and neuronal cell death, including alpha-II-spectrin (αII-spectrin) breakdown products (Ref. 74). UCH-L1 is a neuron-specific enzyme that is highly abundant in neurons. Papa *et al.* compared the levels of UCH-L1 in CSF between patients with severe TBI and an uninjured control group. They examined the relationship between UCH-L1 levels and the severity of brain injury, complications and functional outcomes, confirming that this biomarker can determine the severity of injury in TBI patients (Ref. 72). The study by Brophy *et al.* confirmed the role of UCH-L1 in TBI diagnosis and investigated its kinetic profile in the biofluids of patients with severe TBI (Ref. 75). NSE has previously been confirmed as a biomarker for acute neuronal injury. Consequently, some studies have utilized its levels to assess the impact of organic solvent exposure on neurotoxicity (Ref. 76). Under normal circumstances, NSE levels in body fluids, such as serum and CSF, are minimal. However, during neuronal damage in the brain, NSE levels rapidly rise. Hence, NSE can be utilized for the diagnosis and prognosis of TBI (Ref. 73). αII-spectrin is a key constituent of the cortical membrane cytoskeleton and acts as a primary substrate for calpain and caspase-3, both calcium-dependent proteases. Substantial evidence suggests that αII-spectrin undergoes proteolytic processing by calpain and caspase-3, leading to the generation of specific breakdown products. The study by Cardali and Maugeri further demonstrated that the degradation products of αII-spectrin serve as reliable biomarkers for severe TBI in humans (Ref. 77). Microtubule-associated proteins (MAPs) are essential constituents of the cellular cytoskeleton associated with microtubules. They play a crucial role in promoting microtubule assembly and maintaining stability. Specifically, MAP-2 is a neuronal protein predominantly found in dendrites and axons (Ref. 78). Posmantur *et al.* conducted a study using protein blotting and qualitative immunohistochemistry to examine the acute alterations in cortical MAP-2 immunoreactivity in rats after TBI. The results confirmed a reduction in MAP-2 levels at the site of injury following TBI (Ref. 79). Kitagawa *et al.* examined the distribution of MAP-2 in gerbil brains and confirmed its immunohistochemical reaction as a biomarker for monitoring cerebral ischaemic injury progression (Ref. 80). Alpha-synuclein (α-syn) is a 140-amino acid small protein, and there is abundant evidence supporting its involvement in neurodegenerative diseases, including Parkinson's disease (Ref. 81). The clinical symptoms of long-term survivors of TBI often resemble the signs of brain dysfunction observed in neurodegenerative diseases, including Alzheimer's disease. This suggests that chronic TBI and related neurodegenerative diseases might share common neural circuits. Research by Ikonomovic *et al.* has demonstrated the presence of α-syn aggregates in the cortical tissue of severe TBI patients, providing additional support for the hypothesis that head trauma significantly elevates the risk of developing neurodegenerative diseases (Ref. 82).

MS is an effective method for exploring biomarkers of TBI in injured tissues because of its high sensitivity and specificity (Ref. 83). Jenkins *et al.* conducted a study in mice, employing two-dimensional gel electrophoresis to investigate biomarkers of TBI (Ref. 84). Recently, several studies have utilized target protein identification techniques to screen for novel biomarkers of TBI. Wang *et al.* employed tandem mass tagging (TMT) and ultra-high-performance liquid chromatography-tandem mass spectrometry in a study to identify and quantify differentially expressed proteins in a rat model of TBI. The study findings revealed that human kininogen-1, Protein Kinase A Regulatory Subunit 2A and myelin basic protein were potential biomarkers associated with the time of injury, suggesting their potential as effective therapeutic targets (Ref. 85). Hinson *et al.*'s study demonstrated the feasibility of antibody-free proteomic detection of protein biomarkers in the plasma of TBI patients, in addition to the common antibody-mediated protein biomarker detection methods (Ref. 86). Label-free MS can reflect blood–brain barrier disruption in severely injured patients. Furthermore, using techniques such as western blot and immunocytochemistry, Zhang *et al.* confirmed that TBI patients exhibited elevated levels of caspase-8 mRNA and protein compared with the control group. Additionally, they observed the specific phenomenon of caspase-8 degradation into a 20-kDa fragment protein in TBI patients. TBI patients exhibited increased Fas levels compared with the control group, and this increase was correlated with the relative levels of caspase-8. Fas is a protein located on the cell membrane that belongs to the death receptor family and plays a crucial role in programmed cell death. These findings demonstrate the involvement of Fas and caspase-8 in the cascade of programmed cell death following human TBI, thereby revealing partial mechanisms underlying the progression of severe head and brain injury (Ref. 87). All the biomarkers mentioned in this section are consolidated in Table 3.

Post-traumatic thrombus

Blood circulation normally remains in a fluid state, and when a blood vessel is injured, the clotting system is triggered, leading to the formation of a haemostatic clot that adheres to the blood vessel wall without significantly impairing blood flow within the vessel (Ref. 88). Thrombus can be categorized based on location into arterial, venous and microvascular thrombosis, as well as disseminated intravascular coagulation (DIC). Arterial thrombosis occurs when platelets aggregate within an atherosclerotic plaque. Rupture of the plaque can result in local thrombosis and blockage

Table 3. Biomarkers of TBI and their biological functions

Biomarkers	Biological functions
TNF-α	Causes haemorrhagic necrosis in a variety of tumours
S100B	Low concentrations of S100B have neurotrophic effects, high concentrations of S100B have neurotoxic effects
GFAP	A specific intermediate filament protein found in astrocytes and is upregulated during astrocyte proliferation
UCH-L1	A neuron-specific enzyme that is highly abundant in neurons, determines the severity of injury in TBI patients
NSE	Can be utilized for the diagnosis and prognosis of TBI, associated with acute neuronal injury
αII-spectrin	A key constituent of the cortical membrane cytoskeleton and acts as a primary substrate for calpain and caspase-3
MAP-2	Promotes microtubule assembly and maintains stability
α-Syn	Involved in neurodegenerative diseases such as Parkinson's disease

of blood flow, potentially leading to myocardial infarction or stroke (Ref. 89). The process of arterial thrombosis is illustrated in Figure 2. Venous thrombosis typically develops in the major leg veins, with the clot predominantly composed of red blood cells and fibrin. Unlike arterial thrombosis, venous thrombosis primarily arises from alterations in blood composition, changes in blood flow and/or activation of endothelial cells (Refs 90, 91). Capillaries are tiny blood vessels that are not visible to the naked eye without magnification or assistance (Ref. 92). Microvascular thrombosis can contribute to small vessel disease, which is often associated with chronic hypertension and other risk factors for cardiovascular disease, and may result in microinfarctions in the brain and heart (Ref. 93). DIC is a condition characterized by widespread microvascular thrombosis. Sepsis and endotoxaemia are the primary pathological states associated with DIC. Additionally, DIC can occur as a consequence of severe trauma, such as surgery, which releases 'tissue fragments' into the bloodstream and triggers clotting activation (Ref. 88).

Protein C, a vitamin K derivative, plays a crucial role in anticoagulation. Protein S, an important anticoagulant, acts as a non-enzymatic cofactor for activating protein C (Ref. 94). Protein C inactivates clotting factors Va and VIIIa, thus a deficiency in protein C results in the overexpression and activation of these factors, leading to thrombosis (Ref. 95). When platelets adhere to damaged blood vessel walls, they become activated, triggering the activation of platelet integrin GP IIb-IIIa (αIIbβ3), which binds fibrinogen and vWF to form platelet aggregates or mural thrombus. Simultaneously, damaged vessel walls and activated platelet surfaces accelerate local clotting, stabilizing the thrombus through fibrin formation (Ref. 96). The formation of atherosclerotic plaque in blood vessels leads to narrowing of the vascular lumen, increased blood flow rate, and the generation of a pathological high wall shear rate. Rupture of the narrow plaque cap disrupts the endothelial cell layer and exposes pro-coagulant collagen in the extracellular matrix. These events result in vWF binding and elongation, platelet adhesion and shear-induced

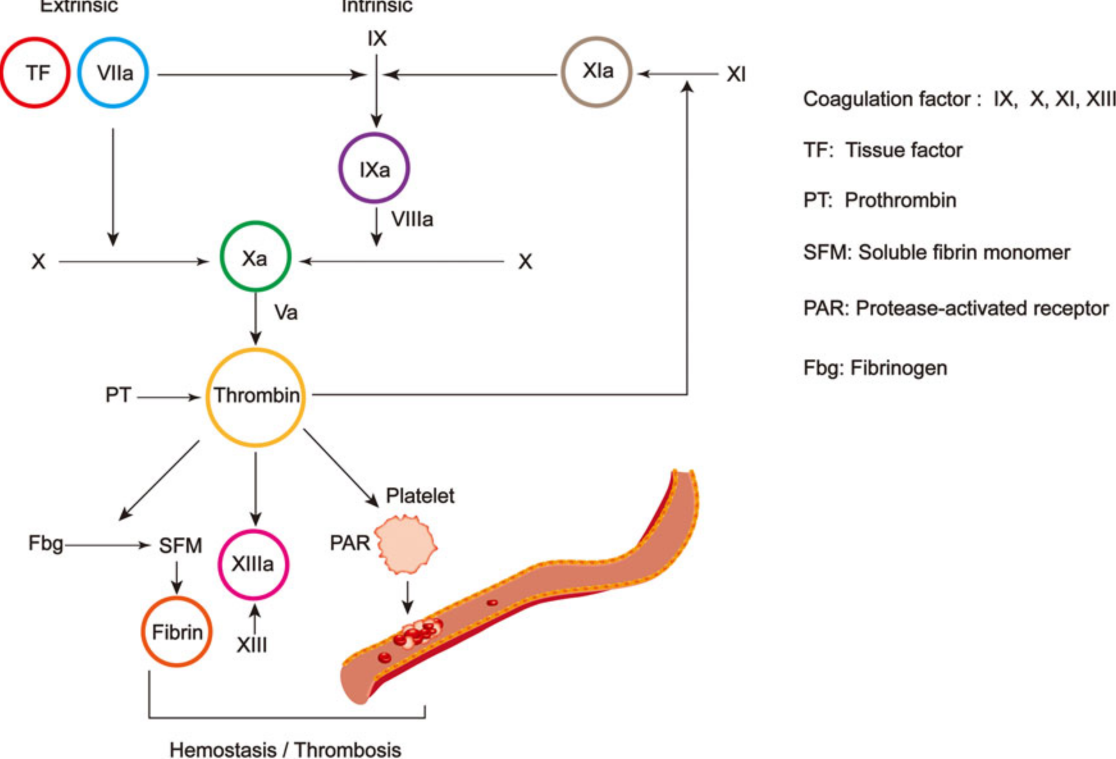

Figure 2. Process of arterial thrombosis – the thrombin cascade reaction. TF triggers coagulation by forming the FVIIa complex, which activates FX and FIX. FXI can activate FIXa. The prothrombin (PT) complex (FVa: FXa) catalyses the conversion of PT to thrombin. Thrombin then stimulates the activity of various proteases and cofactors. Moreover, thrombin cleaves fibrinogen into soluble monomers, which are subsequently cross-linked by FXIIIa. Activation of a protease-activated receptor on platelets leads to the formation of a blood clot.

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platelet aggregation, leading to the formation of occlusive clots and blood flow cessation (Ref. 97). Zhang *et al.* previously described a unique anti-platelet autoantibody in patients with HIV or hepatitis C-associated thrombocytopenia that recognizes the GPIIIa49-66 sequence. After activation by platelet-reducing nicotinamide adenine dinucleotide phosphate oxidase, this autoantibody induces complement-independent thrombocytolysis through the production of reactive oxygen species and peroxides (Refs 98, 99). Subsequently, they discovered a human single chain variable fragment antibody, named A11, which recognizes GPIIIa49-66. This antibody exhibits functional properties similar to patient autoantibodies, preferentially binds to activated platelets and is capable of lysing arterial thrombi in vitro.

Inflammation plays a crucial role in initiating deep vein thrombosis (DVT). Various clinical conditions related to inflammation, including sepsis, systemic infections, cancer, trauma and surgery, are linked to a higher risk of venous thromboembolism (encompassing DVT and pulmonary embolism). Inflammation may be implicated in the pathophysiology of post-thrombotic syndrome, characterized by fibrotic vein damage, incomplete recirculation and valve insufficiency, resulting in elevated venous pressure after DVT (Ref. 100). Because of the involvement of inflammation in thrombosis, organization and degradation processes, numerous inflammation-related factors, including conventional biomarkers (e.g. CRP, IL-6 and other cytokines) and specific markers such as matrix metalloproteinases (MMPs), have been assessed as indicators of thrombotic resolution (Ref. 101). Matrix metalloproteinase 9 (MMP9) inactivation did not impact thrombus formation but had a notable influence on thrombus resolution and the restoration of vascular wall elasticity, resulting in enhanced wall compliance in MMP9 knockout mice. MMP9 inactivation also results in increased macrophage infiltration into the thrombus and a relative reduction in the stiffness of collagen and elastin fibres during thrombus resolution, highlighting the potential of MMP9 as a valuable therapeutic target (Ref. 102). Thromboregulatory protein (THBD) enhances endothelial cell survival and angiogenesis. By means of its epidermal growth factor-like repeat sequence and serine/threonine-rich domain, THBD facilitates thrombin activation of protein C and its cofactor carboxypeptidase-B (Ref. 103). Activated protein C (APC) functions as an anticoagulant in the bloodstream through the proteolytic cleavage of coagulation factors V and VIII. Furthermore, APC exhibits anti-inflammatory properties by binding to endothelial protein C receptor expressed on neutrophils and monocytes (Ref. 103). Therefore, THBD is regarded as a crucial protective factor against thrombosis and inflammatory conditions (Ref. 104).

Post-traumatic ischaemic necrosis

Tissue ischaemic necrosis occurs when tissues and organs do not receive timely blood supply following trauma, leading to necrosis. Ischaemic necrosis can manifest in different body parts, including the heart, brain, digestive tract and bones (Refs 105, 106, 107, 108). Ischaemic necrosis exhibits noticeable variations in occurrence time among different tissues and organs. Tissue ischaemic necrosis primarily occurs in regions with abundant tissue blood perfusion or regions with impaired blood supply. Consequently, severe tissue damage in these areas leads to the destruction of protective mechanisms that maintain blood supply, causing inadequate perfusion and resulting in tissue ischaemic necrosis (Ref. 109). Human tissues rely on adequate blood supply for proper function. Ischaemia directly impairs blood perfusion and normal physiological activities of diverse tissues and organs. Delayed treatment can result in cellular apoptosis, exacerbating inflammation and predisposing to other lesions (Ref. 110).

The target proteins associated with post-traumatic ischaemic necrosis primarily include the following: GFAP, B-cell lymphoma-2 (Bcl-2), receptor-interacting protein kinase (RIPK3), receptor-interacting protein 3 (RIP3), connexin 43 (CX43) and adenine nucleotide translocase 1 (ANTI). GFAP, in addition to serving as a biomarker for TBI, has also been implicated in studies associated with ischaemic necrosis. The peak value of GFAP is correlated with the duration of TBI as well as the degree and maturity of neuronal necrosis. Petito CK and Halaby IA employed immunohistochemistry and optical density analysis to assess the alterations of GFAP in astrocytes, aiming to examine the level of neuronal necrosis in mice following transient ischaemia (Ref. 111). The results demonstrated an initial decline and delayed rise of GFAP in the constructed brain injury model. Additionally, it observed astrocyte swelling and increased transcriptional activity. Moreover, the duration of transient ischaemia had a significant impact on the immunoreactivity of GFAP. According to Kim *et al.*'s research, the Bcl-2 protein, involved in apoptosis, plays a crucial role in protecting neuronal cells with acquired ischaemic tolerance (Ref. 112). The team conducted immunohistochemistry to investigate the cerebral cortex hippocampus region CA1 after gerbils experienced transient global cerebral ischaemia. Necroptosis is a distinct form of programmed necrosis observed in neurological disorders, including cerebral ischaemia. RIPK3 serves as a crucial target in the necroptosis pathway. Fayaz *et al.* employed small molecule compounds to target and suppress the activity of RIPK3, effectively safeguarding neurons during ischaemic stroke (Ref. 113). Ischaemic diseases have been confirmed to be associated with abnormal cell death induced by ischaemic stress. The pathogenesis of ischaemic diseases has been shown to be associated with abnormal cell death induced by ischaemic stress. Lee *et al.* utilized computer simulation interaction analysis, and combined experimental approaches to provide initial evidence that phosphorylated death-associated protein (Daxx) plays a crucial role in mediating ischaemic cell death via RIP3 (Ref. 114). Fas-associated factor 1 (FAF1) is a protein known for its involvement in apoptosis, but its role in the pathogenesis of ischaemic diseases remains unclear. In their study, Yu *et al.* established a mouse model of retinal ischaemia to investigate the potential association of FAF1 with the pathogenesis of ischaemic diseases (Ref. 115). The study revealed that FAF1 plays a crucial role in the development of ischaemic retinal damage and may be implicated in the pathogenesis of retinal ischaemic diseases. Xi *et al.* investigated microRNA-206 (miR-206) regulates the CX43 extracellular signal-regulated protein kinase (ERK)1/2 pathway whether involved in the occurrence of glucocorticoid-induced avascular necrosis of the femoral head (Ref. 116). The results demonstrated that miR-206 down-regulates its target protein CX43, leading to the inhibition of the ERK1/2 signalling pathway and osteogenic differentiation. Moreover, miR-206 is involved in the development and repair processes of steroid-induced necrosis of the femoral head. Wang *et al.* investigated the involvement of miR-2861 and ANTI in the regulation of cardiac cell necrosis during myocardial ischaemic necrosis (Ref. 117). They found that ANTI not only inhibited h202-induced cardiomyocyte necrosis but also antagonized myocardial necrosis in a mouse model of ischaemia/reperfusion. Biomarkers of trauma and their biological functions are shown in Table 4.

Target protein identification techniques

Proteins are vital constituents of human tissues and cells, participating in nearly all biological processes and functions. Proteins exert regulatory functions in the human body, serving as hormones, enzymes and signalling molecules that play a critical

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Table 4. Biomarkers of post-traumatic ischaemic necrosis and their biological functions

Biomarkers	Biological functions
GFAP	Correlated with the duration of TBI as well as the degree and maturity of neuronal necrosis
Bcl-2	Plays a crucial role in protecting neuronal cells with acquired ischaemic tolerance
RIPK3	Serves as a crucial target in the necroptosis pathway
RIP3	Daxx plays a crucial role in mediating ischaemic cell death via RIP3
CX43	One of the important regulatory substances that affect the speed and quality of wound healing
ANTI	Involved in regulation of cardiac cell necrosis during myocardial ischaemic necrosis

role in governing human metabolism, immune response and cellular signal transmission (Ref. 118). They contribute to maintaining a stable physiological state in the body and actively participate in the body's adaptation and response to internal and external stimuli. Moreover, abnormal levels or structural alterations of proteins can indicate the presence of diseases or pathological conditions (Ref. 119). For instance, abnormal levels of certain proteins, either excessive or deficient, may be associated with diseases including tumours, inflammation, cardiovascular disorders and cerebrovascular diseases. Through the detection and analysis of protein alterations, it facilitates disease diagnosis, assessment of disease severity and monitoring of treatment efficacy.

Proteomics is now recognized as a more suitable approach than genomics for comprehending gene function since it examines the final output of the genome. Target protein identification technology relies on sophisticated experimental methods and analytical techniques, including MS, electrophoresis and small interfering RNA blocking, among others. This technology aids in the identification of target proteins linked to specific diseases or biological processes, allowing for the determination of their features, such as expression level, localization, structure and function.

Mass spectrometry

MS is a method used to study protein complexes, providing insights into subunit stoichiometry, composition and protein–ligand and protein–protein interactions. MS can be employed to determine the amino acid sequence of peptides and characterize various post-translational modifications, including phosphorylation and glycosylation. Additionally, it enables absolute and relative quantification of proteins, allowing the identification and quantification of thousands of proteins from complex samples (Ref. 120). Its speed, high sensitivity and versatility have made it an indispensable tool for peptide and protein analysis. MS consists of three basic components: an ion source, a mass analyzer and a detector. An ion source is utilized to ionize the species under analysis, and the resulting ions are subsequently transported to the mass analyzer by a magnetic or electric field. Two commonly employed ionization methods are electrospray ionization (ESI) (Ref. 121) and matrix-assisted laser desorption ionization (MALDI) (Ref. 122). A mass analyzer differentiates ions based on their mass/charge (m/z) ratios. Detectors record the charge induced or current generated as ions pass by or strike a surface, and a computer graphically displays the resulting signal as a mass spectrum.

MS is classified into ESI-MS and MALDI-MS based on distinct ionization methods. Prior to performing MS analysis, ESI employs electrical energy to facilitate the migration of ions from the solution to the gas phase. ESI converts the test sample into ions, then disperses finely charged droplets, generating an aerosol by subjecting the liquid to high pressure (Ref. 123). MALDI employs matrices that absorb laser energy to generate ions from macromolecules, thus preventing thermal decomposition of heat-sensitive compounds caused by rapid heating (Ref. 124). MALDI method comprises three steps. In the first step, a sample is mixed with an appropriate matrix material and coated on a metal plate. Subsequently, the sample is irradiated by a pulsed laser, resulting in ablation and desorption of both the sample and matrix material. Finally, the analyte molecules traverse a thermal plume of ablative gas, where they become protonated or deprotonated and ionized, before entering a mass spectrometer for analysis (Ref. 125).

In protein analysis, MS is commonly coupled with liquid chromatography. The protein mixture is subjected to trypsinization, resulting in the generation of short peptide fragments. These fragments are then separated by chromatography and introduced into a mass spectrometer. The ion source of the mass spectrometer ionizes the sample, charging the peptides, and determines their relative molecular mass by recording the m/z ratio and charge of the ionized peptides. The peptides are then further fragmented to obtain fragment ion information. By combining these two sets of information, m/z ratio spectra can be generated, which can be compared with protein databases to identify proteins corresponding to the peptides in the sample (Ref. 126).

Figure 3 illustrates the protocol for identifying target proteins through MS. Tissue or cell samples were collected and subjected to sample lysis and protein extraction using SDT buffer (4% sodium dodecyl sulphate (SDS), 100 mM Tris-HCl, pH 7.6). Protein quantification was then conducted using a bicinchoninic acid protein assay kit. The proteins were digested into small peptide fragments using trypsin. High-performance liquid chromatography was employed to separate the protein samples. Subsequently, the separated protein samples were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS). The acquired MS data were analysed and interpreted. Protein identification and functional annotation were conducted utilizing suitable bioinformatics tools and databases, including UniProt, NCBI, etc.

Cross-linking MS captures the 3D structure of proteins by covalently linking residues that are in close spatial proximity. The proteins were subsequently identified using MS and database searches based on these structures. In a typical cross-linking MS experiment, proteins are cross-linked in solution, digested into peptides using a protease (typically trypsin) and subsequently analysed by LC-MS/MS in data-dependent acquisition mode (Ref. 127).

Currently, MS is a pivotal technology for screening protein markers and drug therapeutic targets. Hinson *et al.* employed antibody-free proteomics to identify protein biomarkers in the plasma of patients who experienced severe trauma. MS detected the non-specific upregulation of vascular adhesion molecule 1 and MMP9, which serve as protein markers reflecting blood–brain barrier disruption, in severely injured patients (Ref. 86). Peng Zhang *et al.* employed isobaric tag for relative and absolute quantification in conjunction with LC-MS/MS to measure changes in brain protein expression in rats with diffuse axonal injury (DAI). They successfully identified and quantified a total of 1858 proteins, including four novel biomarkers (citrate synthase, synaptosomal-associated protein 25, microtubule-associated protein 1B and Rho-associated protein kinase 2), which offer potential insights into the pathophysiological mechanisms underlying DAI (Ref. 128). Junjie Zhang *et al.* employed surface-enhanced laser desorption/ionization-time of flight mass spectrometry for the screening of differentially expressed proteins in serum samples from Wilms tumour

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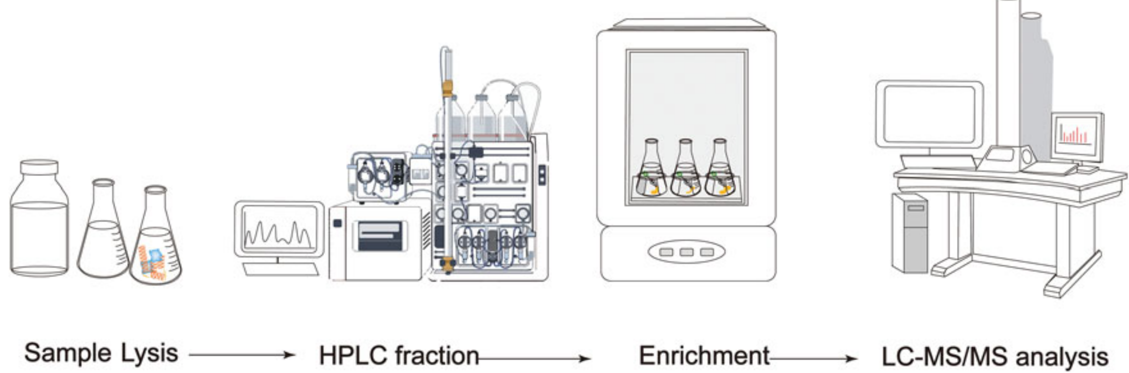

Figure 3. Process of quantifying proteins through MS.

patients. They observed an increased level of thioredoxin 1, an inflammatory factor associated with post-traumatic stress, suggesting its potential role in post-traumatic stress in Wilms' tumours (Ref. 129).

Electrophoresis

Gel electrophoresis

Gel electrophoresis encompasses several techniques: one-dimensional PAGE, two-dimensional PAGE (2D-PAGE), isoelectric focusing (IEF) electrophoresis and non-denaturing gel electrophoresis. In one-dimensional gel electrophoresis, the migration rate of proteins is determined by the pore size, protein charge, size and shape of the gel, leading to their separation (Ref. 130). 2D-PAGE is a commonly used technique in proteomics research (Ref. 131). IEF is a widely used technique for separating proteins based on their isoelectric point pH (referred to as pI). It is employed for both analytical and preparative protein separations (Ref. 132). IEF is an electrophoretic technique in which amphiphilic molecules separate as they migrate through a pH gradient (Ref. 133). One major limitation of sodium dodecyl sulphate PAGE (SDS-PAGE) is that it intentionally denatures proteins prior to electrophoresis, resulting in the inability to detect enzyme activity, protein cofactors and protein-binding interactions (Ref. 134). In contrast to denaturing methods, native gel electrophoresis does not employ charged denaturants such as SDS, allowing the preservation of the native protein structure. Consequently, the migration of proteins is determined by their inherent charge (Ref. 135).

The general procedure for detecting trauma-related target proteins through gel electrophoresis is as follows: utilize the identical methods for protein extraction and quantification as employed in MS. Then, prepare polyacrylamide gel and the corresponding electrophoresis buffer. Conduct necessary pre-processing steps on the proteins, such as reduction, alkylation, etc. Subsequently, the prepared protein samples were loaded into the gel slots, connect the slots to the electrophoresis system and apply a suitable voltage to separate and mobilize the proteins within the gel. If desired, stain the gel using a dye, such as Coomassie brilliant blue, to visualize the protein bands. Analyse the gel electrophoresis image using suitable image analysis software, and determine the migration position of the target proteins by referencing known molecular weight markers. Finally, perform identification and annotation of the target proteins.

Capillary electrophoresis

Capillary electrophoresis (CE) is a sensitive and versatile technique that encompasses a series of electrophoretic separation methods performed in capillaries with submillimetre diameters, as well as microfluidic and nanofluidic channels. These methods include capillary zone electrophoresis, capillary isoelectric focusing, capillary gel electrophoresis (CGE), affinity capillary electrophoresis (ACE), capillary isotachophoresis and micellar electrokinetic chromatography (Ref. 136). CGE is a widely employed technique for protein separation. CGE offers several advantages over traditional SDS-PAGE, such as on-column detection, automated operation, superior separation capability and precise quantification of proteins and determination of molecular weights (Ref. 137). ACE is a specialized form of CE that utilizes intermolecular binding interactions to investigate protein–ligand interactions (Ref. 138).

Immunological methods

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a highly sensitive and specific biochemical analytical method used for detecting, quantifying or qualitatively analysing analytes.

Here are the basic steps for ELISA: (1) coating: the first step involves coating a microplate with the substance to be detected. This is typically done by immobilizing the antigen on the surface of the plate. (2) Blocking: after coating, the plate is blocked to prevent non-specific binding. This involves adding a blocking agent (e.g. bovine serum albumin or milk) to cover any remaining uncoated surfaces on the plate. (3) Primary antibody incubation: the sample, which may contain the target molecule (e.g. a specific protein), is added to the wells of the microplate. If the target molecule is present, it will bind to the immobilized antigen. (4) Washing: the plate is then washed to remove any unbound or non-specifically bound substances. (5) Secondary antibody incubation: a secondary antibody, which is specific to the primary antibody and is conjugated with an enzyme (e.g. horseradish peroxidase or alkaline phosphatase), is added. This secondary antibody binds to the primary antibody that is already bound to the target molecule. (6) Washing: again, the plate is washed to remove any unbound secondary antibody. (7) Enzyme reaction: a substrate specific to the enzyme is added. If the enzyme is present (because the secondary antibody is bound), it will catalyse a reaction that produces a detectable signal, often a colour change. (8) Measurement: the intensity of the colour change is proportional to the amount of the target molecule present in the sample. This can be measured using a spectrophotometer.

ELISA has various variations depending on the specific application, including direct ELISA, indirect ELISA, sandwich ELISA and competition ELISA (Fig. 4). It is a highly sensitive and specific technique extensively employed in clinical laboratories, research and diagnostics to detect and quantify various biological substances.

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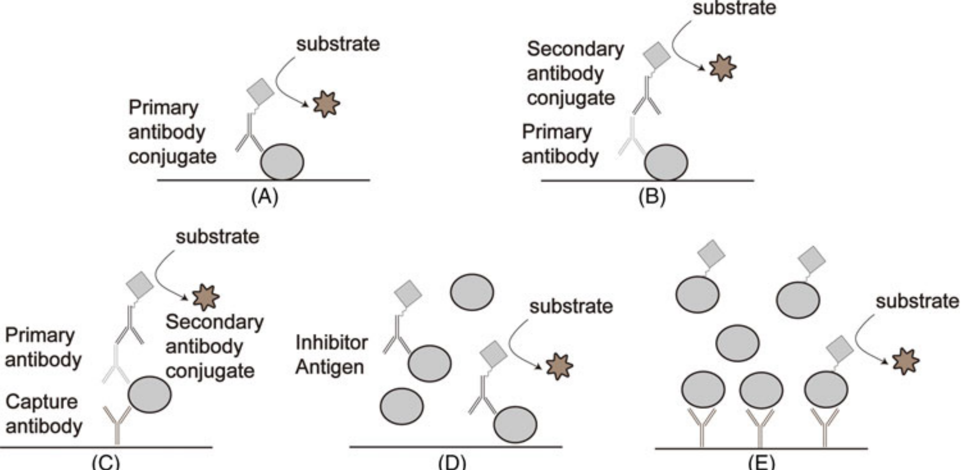

Figure 4. Popular ELISA formats: (A) direct ELISA; (B) indirect ELISA; (C) sandwich ELISA; (D) competitive ELISA with labelled antibody and (E) competitive ELISA with labelled antigen.

In recent years, there has been significant progress in the development of enzyme-linked immunosorbent technology, making it a valuable research tool in immune studies, immune diagnosis and immune-related quality control detection across different industries.

One of the advantages of ELISA is that it can be performed without the requirement of complex or expensive equipment. The analyte in ELISA can refer to a specific substance, specific protein or even a complex mixture comprising multiple proteins, such as biomolecular complexes (Ref. 139).

Cathepsin X, a lysosomal cysteine-type carboxypeptidase, is mainly found in monocytes and macrophages. It can be released into circulation either because of the composition of immune cells or regulated secretion. Plasma levels of cathepsin X show a significant increase within the initial 72 h after trauma. ELISA can detect cathepsin X levels in both white blood cells and plasma samples from both healthy volunteers and patients with multiple injuries. The high sensitivity and specificity of this method are valuable for further assessing cathepsin X as a marker for diagnosing and recovering from inflammatory diseases (Ref. 140).

Western blot

Western blotting (WB) is a widely employed technique for protein analysis, involving the separation of native or denatured proteins through gel electrophoresis, their subsequent transfer onto protein-binding membranes and the detection of target proteins using specific antibodies (Ref. 141). This procedure involves multiple steps, including: (a) sample preparation, which entails protein extraction and measurement of protein concentration from cell or tissue lysates; (b) electrophoresis on an SDS polyacrylamide gel to separate proteins based on size; (c) immobilization of the separated proteins onto nitrocellulose or polyvinylidene fluoride membranes; (d) blocking of non-specific proteins on the membrane; (e) detection of target proteins using specific primary antibodies; (f) incubation with labelled chemiluminescent or fluorescent molecule-conjugated secondary antibodies; (g) detection of signals indicating antigen/antibody binding and (h) densitometry analysis of protein bands of interest using software (Ref. 142).

Following these basic steps, researchers can identify and quantify specific proteins in a complex mixture, providing valuable information about gene expression and protein levels in biological samples. Consequently, WB can be used to isolate and identify proteins in scientific and biomedical research, thus aiding in the diagnosis of various diseases.

WB technique is a well-established molecular biology method that has been utilized for over 30 years. It offers high selectivity and sensitivity in protein detection, making it a valuable tool for identifying target proteins in diverse samples. Recent studies indicate that the complement and apoptosis systems in cells are activated as part of a danger-sensing and signalling cascade following severe tissue trauma reactions, particularly in patients with multiple injuries. The early activation of the complement cascade and excessive production of the potent allergic toxin C5a are demonstrated to modulate the immune response following trauma. Huber-Lang *et al.* (Ref. 143) employed western blotting to elucidate the interaction between the central complement component C5 and the pro-apoptotic aspartic protease cathepsin D. In vitro co-incubation of C5a and cathepsin D led to a progressive increase in the concentration of C5a over time. However, when inhibited by the aspartic protease inhibitor pepstatin A, these findings offer in vitro evidence that the aspartic protease cleaves C5, leading to the production of functional C5a. This represents a novel pathway for complement activation.

Lateral flow immunoassays

Lateral flow assay (LFA) is a strip-based platform used for rapid detection and quantification of analytes in complex mixtures. It involves applying the sample to the detection device and obtaining the test result within 5–30 min. The basic principle involves the liquid sample (or extract) containing the target analyte moving across different regions of the polymer strip through capillary action. Specific regions of the strip can bind to molecules that interact with the analyte, and a labelled analyte-specific molecule can also be present. The test result is visually distinguishable (Ref. 49).

Lateral flow immunoassays (LFIAs) operate on a simple and rapid principle. Test strips typically consist of four components: a sample pad, a binding pad, a detection pad and absorbent paper. The process of LFIAs is shown in Figure 5. It begins when a liquid sample (e.g. blood, urine, saliva) is applied to the sample pad. Then, the sample moves to the conjugate pad, which contains particles (commonly gold or coloured latex beads) coated with molecules that can bind to the target analyte in the sample. The sample continues to flow through the reactive membrane, which is a strip or membrane with immobilized capture reagents. These capture reagents can be antibodies or other molecules that specifically bind to the target analyte. Finally, the mixture reaches the absorbent pad, which helps wick the liquid through the membrane.

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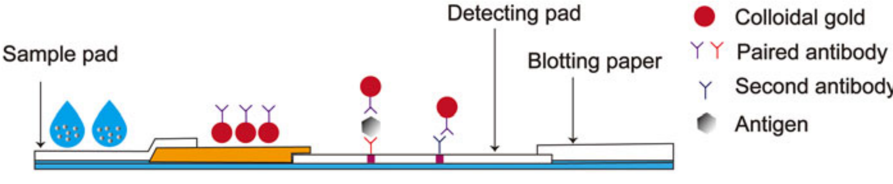

Figure 5. Schematic diagram of LFIAs.

The cost-effective development and simple production of LFA enable its broad application in various rapid detection fields. It can be utilized for qualitative and quantitative detection of specific antigens, antibodies, gene amplification products as well as diverse biological samples (Ref. 49).

Raman spectroscopy

In 1928, the Indian scientist C. V. Raman was inspired by the Compton effect and discovered the phenomenon of inelastic light scattering, now known as the Raman effect. This effect is universal in all types of molecules, whether they are in the form of gas, liquid or solid. When a sample is illuminated by visible monochromatic light, most of the light is absorbed, while a portion of it is scattered. During this process, the incident photons interact with the molecules in the sample, resulting in elastic collisions. The absorbed energy causes the molecules to transition to an unstable virtual energy state, followed by the release of energy and a photon during the relaxation process. When the released energy matches the absorbed energy, the molecule returns to its original energy level, resulting in the scattered light having the same frequency as the incident light. However, only the direction of the scattered light changes, and this scattering phenomenon is referred to as Rayleigh scattering. In the case of inelastic collisions between photons and molecules, the direction of the photon changes, and energy exchange occurs between them. After the energy release, the molecule does not return to its initial energy level, resulting in an energy difference between the final and initial states. This difference leads to the phenomenon known as Raman scattering, where the energy lost by the electron is not equal to the absorbed energy, causing the incident frequency to differ from the scattering frequency (Ref. 144). Raman scattering can be further classified into Stokes Raman scattering, which occurs when the scattering frequency is lower than the incident frequency, and anti-Stokes Raman scattering, which occurs when the scattering frequency is higher than the incident frequency. When the two frequencies are equal, the scattering is referred to as Rayleigh scattering (Ref. 145).

Raman spectroscopy, named after C. V. Raman, is a non-destructive method based on inelastic vibration scattering that is used to detect molecular vibrations. Each molecule possesses unique chemical bonds, resulting in distinct vibrational modes and corresponding energy changes, known as Raman shifts. This characteristic Raman shift provides a specific fingerprint for the biochemical composition and structure of tissues. Consequently, each molecule has its own characteristic Raman spectrum, which allows for qualitative analysis by determining rotational energy levels. Additionally, the intensity of the Raman line can be used for quantitative analysis to determine molecular concentration (Ref. 146). Raman spectroscopy is highly sensitive, non-invasive and effective in identifying target proteins. It can be used for in vivo molecular imaging and in vitro detection of metabolites and protein biomarkers (Ref. 147). In Raman spectroscopy, a common method involves collecting Raman spectra by irradiating the sample with a laser beam through a microscope objective. The scattered Raman light is then reflected back to the optical path through the microscope objective, and finally, the light signal is converted into an electrical signal to generate Raman spectra.

Raman spectroscopy has been employed to analyse differences in target proteins between healthy and sick samples. For instance, a comparative analysis using Raman spectroscopy was conducted on mice induced with sepsis and uninfected mice. The results revealed a significant increase in haemoglobin content in mice with sepsis compared with normal mice (Ref. 148). The process is illustrated in Figure 6. Additionally, a large amount of haemoglobin was released from red blood cells, and the haemoglobin content was positively correlated with the mortality rate of sepsis patients (Ref. 149). Raman spectrum analysis has also identified platelet-derived growth factor-BB as a key biomarker for distinguishing patients undergoing coronary intervention (Ref. 150). Raman spectroscopy offers several advantages, such as eliminating the need for sample preparation, applicability to a wide range of objects and non-intrusiveness. However, the weak Raman signal resulting from the small Raman scattering cross section of molecules remains a challenge in Raman spectroscopy. The Raman scattering cross section can be understood as the probability of collision between photons and molecules. Currently, plasmon-enhanced Raman scattering (PERS) has emerged as an effective method to enhance Raman signals. One common mode of PERS is surface-enhanced Raman scattering (SERS), which enables the identification of biomolecules such as target proteins and nucleic acids. The interaction of biomolecules bound to metal surfaces alters the spectral characteristics, thus enhancing the Raman signal (Ref. 151). In this case, the vibrational energy levels of molecules are primarily excited by the collective oscillation of conduction electrons in metals. When appropriately excited, nanostructures generate a super-strong electromagnetic near-field near their surfaces. Tip-enhanced Raman scattering (TERS) is another mode of PERS that allows for the identification of specific proteins. This technique involves scanning a laser beam on the tip, fixing the laser at the point of highest enhancement and using this arrangement for further measurements. The measured parameter value depends on the spectral quality associated with the electromagnetic field enhancement of a specific tip. TERS requires special sample preparation and substrate (Ref. 152).

SERS and TERS are two different PERS techniques with differences in sample preparation. SERS usually uses metal substrates with good surface enhancement effects to enhance the Raman signal of the sample. The sample to be tested is typically added directly as a solution onto the cleaned and treated metal substrate, where it can be adsorbed through interaction with the metal surface. The prepared SERS samples can be characterized using techniques such as scanning electron microscopy and atomic force microscopy. In a study, SERS technology combines aptamer sensors for sensitive detection of protein biomarkers. A nanocomposite with a special structure was designed, consisting of flower-like silver nanoparticles and a magnetic material. Specific DNA aptamers were used to selectively and effectively modify the surface of the nanocomplex for capturing cTnI target proteins. Binding of the target

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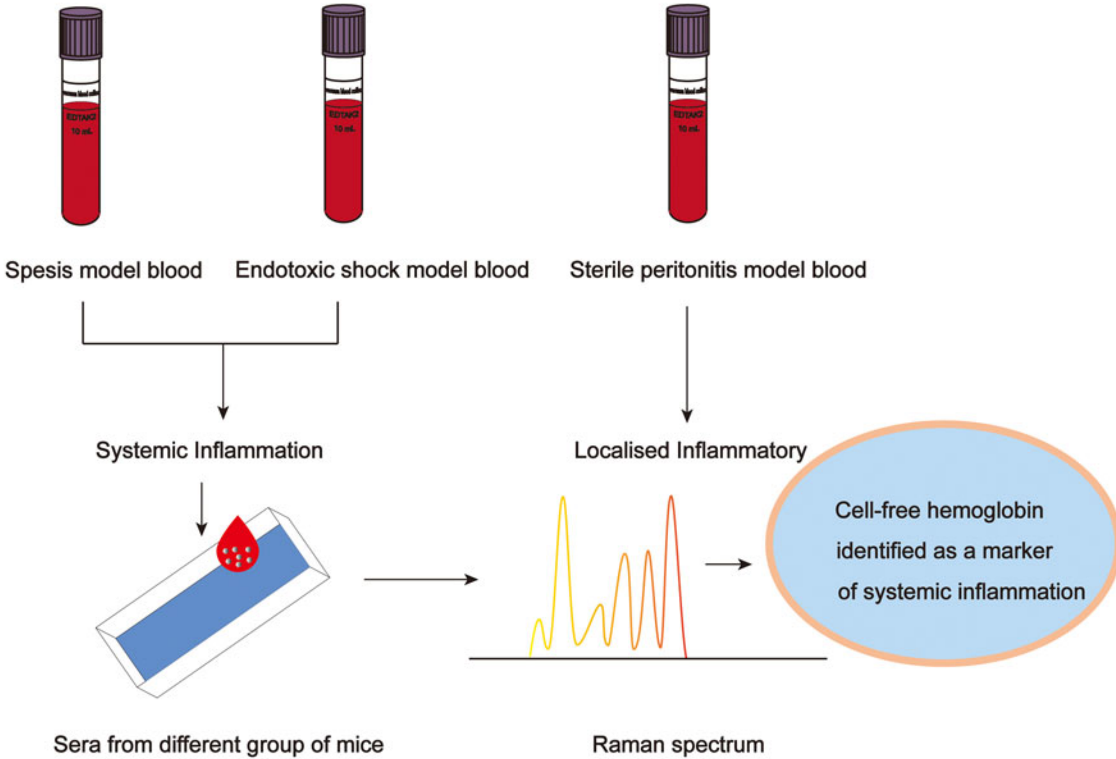

Figure 6. Raman spectroscopy revealed cell-free haemoglobin as a marker for systemic inflammation.

protein to the nanocomplex triggers the SERS effect, generating a specific Raman signal. The design of this SERS adaptor construct provides a novel approach to detect target proteins relevant for trauma diagnosis (Ref. 153).

TERS requires special nanoprobes, typically with metal nanoparticle modifications. These nanoprobes have a fine-pointed geometry and produce a local electromagnetic field enhancement effect. The sample to be tested is typically positioned near the tip of the nanoprobe to establish contact with the probe surface. Local electromagnetic field enhancement can be achieved by adjusting parameters such as the distance and position of the probe and sample, scanning the sample surface with movement of the probe and laser beam while recording Raman signals. TERS technology has the potential to provide information on protein structure, interactions and denaturation states in protein studies.

For instance, TERS technology can be utilized for studying the molecular structure and structural changes of proteins. Collecting TERS spectral data from protein samples allows the extraction of vibrational information about the protein, including vibrational frequency and mode of specific amino acid residues. This information enables researchers to deduce the protein's secondary structure, folding state and potential conformational changes.

TERS technology can be utilized for studying protein–ligand interactions, including those involving drug molecules. By bringing the needle tip into contact with a sample containing the protein and ligand, TERS can provide Raman signals related to the interaction interface. This capability aids researchers in comprehending the interaction mechanism, binding site and binding strength between the protein and ligand. Furthermore, TERS technology enables the investigation of protein denaturation processes and folding states. By monitoring Raman spectral changes in protein samples under varying environmental conditions (e.g. temperature, solvent, pH), TERS facilitates researchers' comprehension of protein stability, structural alterations and changes in folding states (Ref. 154). These applications enhance the potential of Raman spectroscopy for detecting target proteins in wounds.

Summary and outlook

Target protein identification technology holds immense potential for development and allows for in-depth investigation into alterations in body proteins following trauma. By employing techniques such as MS, gel electrophoresis and others, we can detect specific protein markers generated following trauma. These markers can serve as indicators for diagnosing and predicting post-traumatic complications. Moreover, the application of target protein identification technology aids in unravelling the mechanisms involved in wound development and identifying potential targets for therapeutic intervention.

Target protein technology can be integrated with multiomics approaches. For instance, integrating it with genomics research can enhance our understanding of the molecular mechanisms underlying post-traumatic complications. Studies have demonstrated that the progression of sepsis following trauma can vary based on differences in the gene expression profiles of patients. Integrating it with pharmacogenomics research enables us to comprehend an individual's drug response and tolerance. Through analysis of individual genetic variations, it becomes feasible to predict a patient's response to a specific drug and the likelihood of adverse effects, facilitating the development of personalized treatment plans. Additionally, bioinformatics plays a crucial role in trauma management by offering efficient methods for large-scale data processing and analysis. This enhances the identification efficiency of target proteins and novel biomarkers and accelerates their discovery. Moreover, it aids in constructing predictive models to facilitate the development of optimized treatment strategies.

As technology continues to advance and various fields integrate their technologies, we anticipate the emergence of precise and personalized methods for diagnosing and treating trauma. These advancements will significantly diminish the impact of trauma on patient survival.

Abbreviations. 2D-PAGE, two-dimensional PAGE; ACE, affinity capillary electrophoresis; ALI, acute lung injury; Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; ANTI, adenine nucleotide translocase 1; APC, activated

protein C; ARDS, adult respiratory distress syndrome; Bcl-2, B-cell lymphoma-2; CC-16, Clara cell protein 16; CE, capillary electrophoresis; CGE, capillary gel electrophoresis; CRP, C-reactive protein; CSF, cerebrospinal fluid; CX43, connexin 43; DAI, diffuse axonal injury; Daxx, death-associated protein; DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; IEF, isoelectric focusing; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated protein kinase; ESI, electrospray ionization; FAF1, Fas-associated factor 1; GFAP, glial fibrillary acidic protein; GP, glycoprotein; HDL, high-density lipoprotein; ICU, intensive care unit; IGFs, insulin-like growth factors; IGFBP7, insulin-like growth factor-binding protein 7; ILs, interleukins; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8; LC-MS/MS, liquid chromatography tandem mass spectrometry; LFA, lateral flow assay; LFIAs, lateral flow immunoassays; MALDI, matrix-assisted laser desorption ionization; MAPs, microtubule-associated proteins; miR-206, microRNA-206; MMPs, matrix metalloproteinases; MMP9, matrix metalloproteinase 9; MS, mass spectrometry; m/z, mass/charge; NSE, neuronal-specific enolase; PAGE, polyacrylamide gel electrophoresis; PBMCs, peripheral blood mononuclear cells; PCT, procalcitonin; PERS, plasmon-enhanced Raman scattering; PT, prothrombin; PTX3, pentraxin 3; RAGE, receptor for advanced glycation end products; RIP3, receptor-interacting protein 3; RIPK3, receptor-interacting protein kinase; S100B, S100 calcium-binding protein B; SDS, sodium dodecyl sulphate; SDS-PAGE, sodium dodecyl sulphate PAGE; SERS, surface-enhanced Raman scattering; SP-D, surfactant protein-D; sRAGE, soluble receptor for advanced glycation end products; TBI, traumatic brain injury; TERS, tip-enhanced Raman scattering; TF, tissue factor; THBD, thromboregulatory protein; TNF-α, tumour necrosis factor-alpha; UCH-L1, ubiquitin C-terminal hydrolase-L1; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor; WB, Western blotting; αIIbβ3, IIb-IIIa; αII-spectrin, alpha-II-spectrin; α-syn, alpha-synuclein

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