Chapter

Biomarkers of Diseases: Their Role in Emergency Medicine

Anoop T. Chakrapani

Abstract

Biomarkers have been playing an increasingly significant role in clinical decision making processes worldwide. Numerous studies are being undertaken across the globe in the elusive search for the ideal biomarker for each clinical condition. In the emergency department, where rapid diagnosis of various diseases like acute coronary syndromes, pulmonary embolism, heart failure, sepsis, acute renal failure etc. is of utmost importance, specific biomarkers can expedite the time to diagnosis and treatment. To enumerate, the following biomarkers have proved their worth within the setting of emergency departments across the world. The role of cardiac troponins and CK-MB has been well established in the clinical algorithms to detect myocardial infarction. Newer markers like Heart Fatty Acid Binding Protein (H-FABP), BNP, Pro BNP as well as Ischemia modified albumin (IMA) are coming into the fray in the detection of cardiovascular emergencies, especially in the detection of heart failure. Novel biomarkers like Mid-region Proadrenomedullin (MR-proADM) are found to be useful in sepsis along with Tumour necrosis factor-alpha (TNF-alpha), Interleukins and Presepsin in burns patients. Human neutrophil gelatinase-associated lipocalin (NGAL) levels can detect renal failure much earlier than conventional methods. S100 calcium binding protein B (S100B) has been found to be useful in detection of CNS injury and hence can be used to avoid unnecessary radiation to patients in the form of CT scans. Point of care testing of many of these biomarkers in the Emergency department itself paves way for a revolutionary step in faster emergency care delivery and better patient outcomes.

Keywords: biomarker, emergency medicine, troponin, sepsis, burns, acute kidney injury, head injury

1. Introduction

According to the National Institute of Health (NIH) Biomarkers Working Group, a biological marker (biomarker) is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [1].

Worldwide, biomarkers have been primarily studied specifically in the setting of diseases. Even so, it is imperative to understand the concept that a biomarker in the human milieu can be present as a result of normal physiological functioning and need not be the result of a pathological process always. Analysis of various biomarkers, both quantitatively and qualitatively have resulted in better understanding of physiological as well as pathological processes of the human body. An ideal

biomarker should aid in early diagnosis, help in risk stratification, be able to monitor response to treatment modalities and predict outcomes better than the clinical processes and investigations existing in medical practice at that point of time [2].

In medical practice, biomarkers can be of diagnostic or prognostic value. In the setting of practicing Emergency medicine, diagnostic biomarkers assume greater significance. In the Emergency department, where time is of the essence, diagnostic biomarkers help in quick clinical decision making as well as patient disposition. A biomarker that is able to make a clinical differentiation between two similar disease conditions is highly valuable for an emergency physician. This helps in rapid diagnosis, which leads to faster treatment initiation. Faster the treatment can be initiated, faster the patient movement. A faster TAT (turnaround time) for patients in the ED translates to lesser waiting times in the ED. An ideal biomarker in the Emergency department should have the following characteristics—High diagnostic accuracy which involves a high degree of sensitivity with reasonable specificity, should be reproducible across platforms and should be cost effective to the patient and the clinician.

Numerous biomarkers have been extensively studied for the purpose of clinical utility, but only a few handfuls have proved their mettle in clinical practice. The abundance of biomarker tests available, pose a not so friendly dilemma to the clinician of the present. Understandably, there is still a long way to traverse from the laboratory table to the patient bedside. Numerous trials and research activities are underway across the world in the field of biomarkers. Most of them are still in various phases of preclinical trials and are expected to be available by the patient's bedside in a few years' time.

In modern clinical practice, biomarkers are being used more and more for clinical decision making. The current gamut of biomarkers available in the clinical realm have changed the way we practice medicine. The increase in the use of biomarkers for clinical decision making has expedited the patient disposition to a great extent. Having said that, there is also the other side of the coin which is the increase in the health care cost. The over dependence of clinicians on biomarkers should be viewed with caution as this escalates the overall cost of treatment and the patients will have to bear that burden. The decision to make use of biomarkers in the clinical context should be individualised and targeted to prevent its overuse or abuse [3]. This will put a strain on the already scarce resources in the health care sector. It is always pertinent to remember that—A biomarker should always be evaluated in the clinical context and should never be used as a standalone tool for clinical decision making.

In Emergency medical practice, majority of the biomarker work has been focussed in the field of cardiology, renal failure and sepsis, as early detection and prompt interventions in the early phases of the diseases can significantly alter the natural course of the disease and improve the patient morbidity and mortality. Other areas of focus are hepatic diseases, traumatic brain injury, venous thromboembolism etc., where biomarkers are increasingly being tested for their clinical utility. Therefore, the chapter focuses in detail on these clinically significant biomarkers.

2. Acute coronary syndromes

Acute coronary syndromes have been in the forefront of novel biomarker evaluation research due to its widespread prevalence as well as the need for detection in a time sensitive manner. Almost 20 million patients with symptoms of acute coronary syndromes present to emergency departments in North America and Europe annually [4, 5]. Numerous studies have been performed on various biomarkers, the conventional markers as well as high sensitive variants, and also with respect to

different time frames. In the Emergency department, making a rapid diagnosis of acute myocardial infarction is of utmost importance, as *'time is muscle'*. The earlier a diagnosis of ACS can be made, the earlier revascularisation can be initiated. Early treatment can decrease the morbidity and mortality to a significant extent in case of ACS. At the same time, it is also important to make sure deserving patients are disposed of from the ED as soon as possible in a time dependent manner once ACS is ruled out. This assumes more significance in EDs that receive a high volume of patients and need patients to be either admitted for further workup or discharged in a timely manner. At the same time, discharging patients from the ED always has a risk of patients ending up in an adverse cardiac event. Institutional protocols that include serial biomarker evaluation help in minimising these risks to a great extent.

Historically, biomarkers like LDH (lactate dehydrogenase) and AST (aspartate aminotransferase) were tried in the detection of an acute coronary syndrome especially towards the end of 20th century. Their clinical significance slowly began to decline with the advent of better alternatives as well as lack of specificity. The next in line were the markers with better specificity and sensitivity, namely—the troponins and creatine kinase. As the 21st century is taking its foothold, the scientific community is focussing its attention on these biomarkers for detection of acute coronary events, a leading cause of death worldwide. The research was primarily focussed on Creatine Kinase – MB fraction, which used to be the gold standard of evaluation of ACS. But, that has given way to the newer biomarkers—Troponin I and Troponin T – both conventional and high sensitive, which are being studied extensively across the globe.

2.1 Creatine kinase: MB

Creatine Kinase is an intracellular enzyme with a dimeric molecule which has 3 isoforms—CK-MM (muscle), CK-BB (brain) and CK-MB (myocardium), based on the organ of origin. CK-MB is the isoenzyme fraction which is predominantly seen in cardiac muscle and hence the utility in detecting cardiac muscle damage. This marker is leaked into the systemic circulation from the cellular cytosol due to disruption of the cell membrane as a result of myocardial injury. This marker can be assayed by a clinician to help in the diagnosis of myocardial infarction. CK-MB isoenzyme can be detected in the bloodstream about 4–6 hours after the onset of chest pain. It peaks by 12–24 hrs and returns to baseline by 12–48 hours. This short time window of rise and fall of CK MB is especially useful in detecting reinfarction or infarct extension in a patient in whom the troponin values might be already elevated as a result of an infarct. It is also helpful in identifying complications in patients who have undergone revascularization procedures in the cardiac care unit. The reference values for CK-MB are as follows—males: $\leq$ 7.7 ng/mL and females: $\leq$ 4.3 ng/mL. CK MB assay should always be viewed with a pinch of salt since it is a subunit of the total CK in the system. Abnormal elevations of CK MB can be detected along with an increased level of total CK in cases of traumatic muscle injuries, rhabdomyolysis, myopathies etc. It is worthwhile to note that the normally CK-MB fraction accounts for only 3–5% of the total CK in the body and any increase beyond 30–50% of the total CK should prompt suspicion of abnormal beta subunit synthesis. But, over the past few years, the burden of diagnosis of acute myocardial injury has been shifted on to the shoulders of troponins [6].

2.2 Cardiac troponins

Troponin is a complex protein molecule comprising of three regulatory proteins playing an integral role in the contraction of cardiac and skeletal muscle. These

three subunits are namely—Troponin I (TnI), Troponin T (TnT) and Troponin C (TnC). Each subunit has a unique function. Troponin T binds to the troponin components of Tropomyosin, troponin I inhibits the interaction of myosin with actin and troponin C has the sites for binding of calcium ions to initiate muscle contraction.

Similar to creatine kinase, any cellular injury leads to leakage of the troponins into the systemic circulation thereby providing a window for diagnosis of acute myocardial infarction. Troponins have much higher specificity and sensitivity than creatine kinase. The utility of cardiac troponins especially – Troponin T and Troponin I has been validated in various studies across the world and hence has been incorporated into the diagnostic guidelines of acute myocardial infarction.

Normally troponins are not detectable in the bloodstream due to the minute quantities in open circulation, which is <0.01 ng/mL for Troponin T and ≤ 0.04 ng/mL for Troponin I. After a myocardial injury, elevated troponin levels in the bloodstream can be detected within a period of 4–6 hours, by conventional methods. The reason for this delay in detection has been attributed to the molecular weight and size (21–37 kDa). This can cause clinically significant delay in the diagnosis of myocardial infarction especially in the setting of nonspecific ECG changes. This has led to the advent of high sensitive assays which can detect troponins at much lower levels (at the levels of ng/L) and that too, much earlier than conventional methods. Troponins can be detected as early as 2 hours after the ischemic event by high sensitive troponin assays currently in clinical practice. This also has a caveat, that is, troponin levels can be detected in the circulation even without myocardial injury [4, 5]. Hence a troponin value above the 99th percentile is taken as a diagnostic cut-off for detection of myocardial ischemia. A 20% rise or fall from the baseline within a period of 3–6 hours can confirm the diagnosis of an acute myocardial infarction according to the National Academy of Clinical Biochemistry [6]. It has been recommended by the American college of Cardiology (ACC) that serial values of troponin be considered at 6–9 hr. intervals to rule out NSTEMI [7]. European Society of Cardiology has also reiterated the importance of doing serial assessments of troponins rather than making a clinical decision based on a single value [8]. In a recent large multicentre evaluation in patients with suspected ACS who presented within 8 hours of symptom onset, it was found that it was possible to diagnose ACS with 3-hour marker samples rather than the conventional method of doing serial markers at 6 hour intervals, without losing out on the diagnostic accuracy [9]. Along with clinical evidence of MI, an elevation of troponin level more than 5 times the upper limit compared to the baseline is needed to diagnose a PCI-related MI and more than 10 times the upper limit to diagnose a CABG-related MI [10]. Multiple studies have shown that there is correlation between the levels of troponins and development of adverse cardiac events [11, 12].

Elevated troponin levels may not always indicate myocardial injury [13, 14]. It can also be elevated in non-ischemic conditions as well. A rise/fall in troponin levels are needed to detect acute MI in patients in whom troponins will be elevated otherwise, like renal failure. Even though sensitivity is increased, specificity has come down which may indicate an underlying disease than an acute coronary event. Various causes of nonischemic elevation of troponins are detailed in the below table (**Table 1**). The troponin levels have to be interpreted only in the appropriate clinical setting, failing which the physician may be misled to an alternate diagnosis [15].

2.3 Other contenders

Numerous other biomarkers have piqued the interest of the scientific community to identify acute coronary events more early as well as more precisely. Very few have actually stood on their own when compared to troponin studies. The most

Cardiac	Non Cardiac
• Congestive cardiac failure	• Pulmonary Embolism
• Myocarditis	• Renal failure
• Pericarditis	• Sepsis
• Infiltrative diseases	• Stroke
	• Blunt chest trauma

Table 1.
Examples of non ACS-causes of elevated troponin levels:

• Myoglobin (Mb) P
• Heart Fatty Acid Binding Protein (H-FABP)
• Glycogen Phosphorylase-BB (GP-BB)
• NT-Pro-Brain Natriuretic Peptide (NT-ProBNP)
• D-Dimer
• High Sensitivity C-Reactive Protein (HsCRP)
• Myeloperoxidase (MPO)
• Matrix Metalloproteinase-9 (MMP-9)
• Pregnancy Associated Plasma Protein-A (PAPP-A)
• Soluble CD40 Ligand (SCD40L)

Table 2.
Newer biomarkers for cardiac ischemia.

common drawback being the cost of the investigation as well as availability. Some of the examples are discussed below.

Myoglobin (Mb) peaks within minutes of cardiac ischemia. With the recent advancements for detection of hs-troponin levels, the utility of Mb has come down in the diagnostic algorithm [16]. A few examples of other novel biomarkers of myocardial ischemia/injury that have undergone clinical trials are given below in **Table 2**. They include cardiac intracellular proteins, markers of neurohormonal activation, markers for haemostatic activity, vascular inflammation markers etc. [17]. In this study, the assessment of H-FABP within the first 4 h of symptoms was found to be superior to cTnT for detection of MI. But the reduced specificity of H-FABP is presently limiting its usefulness in clinical practice. Soluble CD40 ligand and choline which are biomarkers signalling the instability of atherosclerotic plaque formation, have been studied, but did not show add any prognostic or diagnostic value to the existing ones in practice. But the other biomarkers they studied along with this, did not show any favourable clinical significance.

3. Cardiac failure

Cardiac failure is a complex process involving a multitude of pathophysiological processes. As a result of this, various biomarkers have been identified which correlate with specific aspects of heart failure. The marker which has made its mark in a clinically significant manner are the natriuretic peptides-B type natriuretic peptide (BNP) and NT pro BNP.

3.1 B type natriuretic peptide (BNP) & NT pro BNP

BNP is secreted from the ventricles as a result of neurohormonal activation due to volume overload and resultant stretching of the myocardial muscle fibres. In patients with left ventricular dysfunction/failure, high plasma levels of BNP and NT pro BNP are specific for elevated filling pressures in the cardiac chambers. This can be used in the clinical context for the diagnosis as well as prognostication of cardiac failure. ProBNP is a 108-amino acid polypeptide precursor which is stored in secretory granules in both ventricles and, to a lesser extent, in the atria. After proBNP is secreted, it is cleaved to the 76-peptide, biologically inert N-terminal fragment NT-proBNP and the 32-peptide, biologically active hormone BNP. BNP is rapidly cleared from the circulation; the plasma half-life being approximately 20 min. No receptor-mediated clearance of NT-proBNP is known to occur, because of which NT-proBNP has a prolonged half-life of 60–120 min. The reference values for NT-proBNP varies widely with age and gender, which can be tricky for the clinician while assessing patients, especially in the elderly population (**Table 3**).

In the multicentre Breathing Not Properly Study [18], using plasma BNP level of 100 pg/mL as cut off, gave a sensitivity of 90%, specificity of 76% and a diagnostic accuracy of 81% which was superior to clinical assessment alone in a series of 1586 patients who presented to the ED with acute dyspnoea. A BNP level < 100 pg/ml or an NT-proBNP level < 300 pg/ml can essentially rule out Acute HF in most cases. When using N-terminal proBNP for the diagnosis of acute CHF, a value of 900 pg/mL has high specificity and sensitivity.

Apart from left ventricular failure, these biomarkers can be elevated in numerous other conditions which can cause myocardial stretch. Patients with right ventricular failure secondary to pulmonary embolism or pulmonary hypertension, valvular heart disease, arrhythmias such as atrial fibrillation, renal failure and advanced age may also have elevated levels of BNP or NT-proBNP [19]. In severe renal failure, the NT-pro BNP value of >1200 pg/mL is needed to make a diagnosis of cardiac failure. A common clinical scenario in which the patient is obese, the pro-BNP values can be falsely lower which can mask cardiac failure and lead to misdiagnosis.

The European Society of Cardiology Task Force has recommended that the algorithm for HF diagnosis should include an NP assay as the first step along with electrocardiography (ECG) and chest X-ray [20]. Biomarkers are not just useful in the diagnostic algorithm, but also in guiding treatment. In a meta-analysis [21] of 2686 patients in 12 randomised trials, the researchers found that the use of cardiac peptides to guide pharmacologic therapy significantly reduces mortality and HF related hospitalisation in patients with chronic HF.

As discussed in the previous section, Troponin I (TnI) also plays an important role in the pathophysiological profile of cardiac failure. Newer markers that have potential to be significance in the future for diagnosis and prognosis in heart failure include high-sensitivity C-reactive protein (hsCRP), uric acid and

Age	Males	Females
≤45 yrs	10–51 pg/mL	10–140 pg/mL
45–70 yrs	10–100 pg/mL	10–206 pg/mL
≥70 yrs	10–138 pg/mL	10–1263 pg/mL

Table 3.
Reference values for NT-ProBNP:

myeloperoxidase (MPO), soluble toll-like receptor-2 (ST2) and soluble fms-like tyrosine kinase receptor-1 (sFlt-1). Recently, a study in which the amount of exhaled acetone is measured has shown promise as a newer non-invasive modality for cardiac failure assessment [22]. A recent study attempted to evaluate the predictive utility of these biomarkers with a multimarker score which included BNP, troponin I and creatinine apart from the above markers. They concluded that a multimarker score significantly improves prediction of adverse events in ambulatory patients with chronic heart failure [23]. But, NACB's practice guidelines on cardiac biomarker testing specifically recommends against routine use of biomarker testing only for risk stratification [24]. The newer entries into this field include galectin-3 [25], MR-proANP (midregion pro–atrial natriuretic peptide) [26], MR-proADM (mid regional pro-adrenomedullin), co-peptin, adiponectin, pentraxin-3, soluble ICAM-1(intercellular adhesion molecule-1), PAPP-A (pregnancy associated plasma protein A) etc.

4. Pulmonary embolism

In a case of suspected pulmonary embolism, laboratory evaluation by biomarker levels is primarily helpful in ruling out the diagnosis in low probability scenarios, rather than ruling in a confirmation of a diagnosis. D-dimer has been in clinical use extensively since the past few decades and the other markers which are increasingly used are troponins, BNP and Ischemia modified albumin (IMA).

4.1 D-dimer

D-dimer is a degradation product produced by plasmin during fibrinolysis. The reference value of D-dimer is ≤500 ng/mL Fibrinogen equivalent Units (FEU). It has very low specificity, but a high sensitivity. Due to the low specificity, a clinical diagnosis of pulmonary embolism requires a strong clinical suspicion. In order to help the clinician in this regard, various scoring systems to assess the probability of making a diagnosis of PE have been devised. Well's criteria and its modified version are among the most commonly used. These scoring systems assist the clinician in assessing the probability of a diagnosis of PE along with the blood levels of the biomarker used. In patients with a low pretest probability of PE as assessed by well's criteria and a negative d-dimer value, the diagnosis of pulmonary embolism can be essentially ruled out without any probability of adverse events happening later [27, 28].

4.2 Ischemia modified albumin (IMA)

Ischemia modified albumin (IMA) is a newer marker that has shown potential as a substitute for D-dimer as it has been found to be better than the latter in a few studies due to its better positive predictive value [29]. The reference value for IMA is ≤0.540 ABSU. In patients with pulmonary embolism, more so in those who develop RV dysfunction, other biomarkers like troponins and BNP are also found to be elevated. This occurs due to the increased pulmonary vascular resistance, pulmonary artery pressure and resultant RV afterload. The elevated troponin levels can pose a dilemma for a clinician who wants to rule out ACS as well in the clinical setting as the symptoms of both the conditions may overlap significantly. The elevated levels of BNP/NT pro-BNP in patients with pulmonary embolism have been found to be associated with increase in risk for complications and 30-day mortality [30].

5. Sepsis

Sepsis is a complex process that stems from a combination of features of a systemic inflammatory response to a known or presumed infection. It is associated with a very high mortality rate around 30% not to mention the significant economic impact on the healthcare system [31]. Sepsis can be viewed as a chain of events in the body as a response to an inciting agent through an inflammatory pathway. This provides clinicians the opportunity to diagnose sepsis early by either picking up the inciting agent or the inflammatory response to the agent. More than 170 biomarkers have been identified as useful for evaluating sepsis [32], which itself points to the fact that none of them can be used as a single marker for accurate diagnosis or prognosis. C-reactive protein, procalcitonin and serum lactate are among the prominent ones used extensively worldwide at present.

5.1 C-reactive protein

C-Reactive protein, one of the most commonly used markers for sepsis, is synthesised in the liver as an acute phase reactant. The normal levels in a healthy adult individual tends to be below 10 mg/L. Depending on the severity, any stress or stimulus can cause an elevation in the CRP levels, even manifold up to 500 mg/L. The levels peak around 36–48 hrs and the plasma half-life is approx. 19 hrs. Although very commonly used as an inflammatory marker, it lacks specificity as it is found to be elevated in numerous conditions like post-operative patients, burns, myocardial infarction and inflammatory/rheumatic diseases as well [33]. It can be elevated even in normal individuals especially in elderly as well as pregnancy. Moreover, even a viral infection can cause a mild increase in the serum levels of CRP, contrary to popular belief. The sensitivity and specificity of CRP as a marker for bacterial infections are 68–92% and 40–67%, respectively [34, 35]. CRP plasma levels have shown to correlate with the severity of infection [36] which makes it a useful marker to assess the response to pharmacological treatment.

5.2 Procalcitonin (PCT)

It is a 116-amino acid polypeptide which is the prohormone of calcitonin. It has a short half-life (25–30 hours), and is encoded by the *CALC-1* gene. PCT is normally produced by neuroendocrine cells, mainly in the thyroid (C-cells), from which calcitonin is derived which is responsible for regulation of calcium metabolism in the body. It is also produced in low amounts in other neuroendocrine cells in the intestine and lungs. The *CALC-1* gene is normally suppressed in non-endocrine tissues. Bacterial infection stimulates *CALC-1*gene transcription in non-endocrine cells [37], leading to increased PCT production which can be detected in the circulation making it a marker for diagnosis of bacterial infection and sepsis. PCT is released from various organs including lung, liver, kidney, pancreas, spleen, colon, and even adipose tissues in infectious conditions. In healthy individuals, the serum PCT levels are <0.1 ng/ml, which increases in response to an infective stimulus. Serum PCT levels begin to rise around 4 hrs after the insult and peaks by 24 hrs. The half-life of PCT is approx. 24 hrs and after the infectious process has started resolving, PCT levels decrease by almost 50% every day [38]. In a systematic review and meta-analysis, PCT with a cut-off median value of 1.1 ng/mL was found to be more specific (specificity - 81%) than CRP (67%) for differentiating bacterial infection among hospitalised patients [39]. PCT also has a sensitivity of 77% which makes it a useful marker for early diagnosis of sepsis [40]. These features make PCT a favourable biomarker to be used for guidance of antibiotic stewardship as well to reduce the ever increasing inappropriate use/abuse of antibiotics [41].

5.3 Serum lactate

Lactate is produced in the body even normally, which gets cleared off rapidly in healthy individuals. But, in cases of sepsis and resultant hypoperfusion, the levels of lactic acid increase when anaerobic metabolism increases in the body. Lactate clearance has been shown in a prominent light in the 'Early goal directed therapy' of septic patients. This indicates that, more than a diagnostic marker, lactate has prognostic significance in patients with sepsis. Recent studies have shown that patients with even a milder increase in serum levels in the range of 2–4 mmol/L were at an increased risk of morbidity and mortality [42]. In a study conducted in an urban academic centre which included 1278 patients with infections, those with lactate levels above 4 mmol/L had higher in-hospital mortality rates than patients with lactate levels less than 2.5 mmol/L (28.4% vs. 4.9%) [43]. The bottom line is, the better the lactate clearance, better the outcome of the patient [44].

5.4 Proadrenomedullin (MR-proADM)

Adrenomedullin (ADM) is a 52-amino acid ringed peptide produced from endothelial cells in cardiovascular, renal, pulmonary, cerebrovascular and endocrine tissues. It is a potent endogenous vasodilator in the human body. ADM is not easily measurable due to its very short half-life of 22 minutes in the circulation, its rapid degradation by proteases, and the formation of complexes with circulating complement factor H [45]. The prohormone of ADM - ProADM can be used as a surrogate marker for this purpose as it is more easily quantifiable, and the tools required for this are available commercially. The mid-regional fragment of proadrenomedullin (MR-proADM) is a marker of endothelial dysfunction/inflammation and therefore can be seen in elevated levels in numerous disease conditions. Pro-ADM has been found to be an independent predictor for adverse outcomes in patients with COPD [46]. It has also been studied in the context of burns, in which it was found to have utility in early recognition of onset of sepsis in burns victims [47]. It is still early days for MR-proADM in routine clinical practice as many studies [48] have failed to demonstrate any added utility with respect to other less expensive parameters presently available. For healthy individuals, the reference values for MR pro ADM is <0.5 nmol/L.

5.5 Other markers of sepsis

Cytokines like TNF, IL-1β and IL-6 are the predominant inflammatory mediators responsible for the initial inflammatory response and the levels correlate with the organ damage and mortality [49]. Similarly, High-mobility group box 1 protein (HMGB1) and Macrophage migration inhibitory factor (MIF) are also found to increase in patients with severe sepsis and septic shock and is correlated with the degree of organ failure [50, 51]. Lipopolysaccharide-binding protein (LBS) is an acute phase protein which increases in sepsis and makes it useful as a diagnostic tool as well as a marker for severity of the disease [52, 53]. Other biomarkers like serum amyloid A, eosinophil count, mannan and antimannan, and IFN-γ-inducible protein 10 also show potential to be of use in the future.

6. Burns

In patients who are hospitalised with burns, sepsis is considered as one of the most important causes for mortality. Biomarkers which can help pick up the onset of sepsis in burn patients in the early phase itself will be useful in the proactive

management of complications. Procalcitonin, Tumour necrosis factor-alpha (TNF-alpha), MR Pro-ADM, Interleukins 6, 8 & 10, Presepsin are among the major ones studied in this context in addition to assessment of single-nucleotide polymorphisms (SNPs) and leukocyte transcriptomes [54].

6.1 Procalcitonin

PCT has been extensively studied in the context of sepsis, but literature regarding studies in burns are much lesser in comparison to other critical conditions. Serum levels of PCT were found to be elevated in patients who developed infections after burns in one of the initial studies done in 1993 which had 9 burns patients included among the 79 general patients enrolled in the study [55]. In a recent meta-analysis of around 12 studies in burns patients led the investigators to believe that PCT has a strong ability to differentiate between patients with sepsis and without sepsis [56]. The study proposed that a PCT value >1.47 ng/mL can prompt the clinicians to initiate early antibiotic therapy to counter the development of sepsis and improve patient outcomes.

6.2 Tumour necrosis factor-alpha (TNF-alpha)

It is a proinflammatory cytokine and has been researched worldwide in various disease conditions among the host of numerous inflammatory mediators. It is produced ubiquitously in the body in response to various stimuli which can be infectious or ischemic in nature. They include endotoxins, complement system activation, hypoxia, ischemia as well as reperfusion [57]. TNF-alpha has been found to be elevated in burns and the values are seen to be higher in patients found to be in sepsis [58]. It has also been shown to have a prognostic value in burns victims. In burns patients who were treated with GM-CSF, the values of TNF alpha were shown to come down gradually as the treatment progressed [59], hence proving its role as a prognostic indicator. Reference value: ≤ 2.8 pg/mL.

6.3 Interleukins

The interleukins (ILs) are a large class of cytokines that promote cell-to-cell interactions and the stimulation of humoral or cell-mediated immune responses. They were initially thought to be produced only by the leukocytes, but have been found to be produced from numerous sources since then. The IL family consists of a huge number of members of which IL-6, IL-8 and IL-10 have been shown to be associated with evaluation of sepsis in burns patients. IL-6 has been found to be elevated in burns patients with sepsis [60]. It not only helps in the early diagnosis, but also has prognostic significance regarding the mortality as the levels have been found to be correlating with the size of the burns [61]. Recently, a meta-analysis of studies done on critically ill patients regarding markers of sepsis found IL-6 to have a high specificity, hence making it a suitable marker to confirm an infectious process [62]. Similarly, studies have found that IL-8 levels in burns patients correlate with the development of sepsis and multi-organ failure resulting in mortality [63]. The authors of the study opined that it can be used as a biomarker for monitoring the morbidity and mortality of burn patients developing sepsis. IL-10, similar to its counterparts, have been shown to have a correlation to development of sepsis in burn patients. Normally, the serum levels increase after the injury and decline later. But a failure to decline over time and being persistently elevated should point towards the development of an infective process and may increase the chances of mortality [64], hence making it a prognostic indicator in burn patients.

Some of the other notable markers pertaining to burns patients are - **Presepsin** which is the soluble form of cluster of differentiation 14 (CD14), a glycoprotein that functions as receptor for endotoxin complexes triggering signal transduction pathways implicated in systemic inflammation. In a study conducted on burns patients, Presepsin elevation preceded the elevation of CRP and PCT by 1 day as a marker of sepsis [65]. Several individual studies have reported the diagnostic accuracy of presepsin (sCD14-ST) for sepsis, but the results are inconsistent. Presepsin is an effective adjunct biomarker for the diagnosis of sepsis, but is insufficient to detect or rule out sepsis when used alone [66]. **Single-nucleotide polymorphisms (SNPs)** which are variations in a nucleotide at a specific chromosome location and has been linked to sepsis susceptibility and differences in prognosis in burns patients [67]. Gene-expression patterns like the **leukocyte transcriptome** shifts towards increased expression of genes involved in innate immunity and the inflammatory response and has been noted in burns patients [68]. But, most of these markers are not useful in day to day practice and have been limited to research settings at present. Nonetheless, the possibility of these markers making way into the clinical domain in the future cannot be ruled out.

7. Acute kidney injury

Conventionally, renal function tests which include serum levels of creatinine, urea and assessment of glomerular filtration rate are the methods used to quantify renal diseases. But, these conventional methods have a huge drawback. There is an unacceptable high time lag between the onset of tissue injury and derangement of these biochemical values. This hinders any active reno-protective interventions that may be initiated promptly. Moreover, the serum levels of these markers vary widely even in healthy individuals as it depends on various physical factors like age, gender, muscle mass etc. This has led the medical community to look for alternatives. Human neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18), cystatin C, clusterin, fatty acid binding protein, and osteopontin are the prominent ones that have been studied and NGAL has been the most prominent one of the lot.

7.1 Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophil Gelatinase-associated Lipocalin (NGAL) is a protein that is expressed in neutrophils and has a role in innate immune response as well as repair and reepithelialisation in the kidney. In patients with acute kidney injury, ischemic or nonischemic type, plasma/urine levels of NGAL have been found to be elevated. (>50 μg/L)[69]. Both urinary and plasma levels of NGAL were found to increase by more than 10-fold within 2–6 hours of cardiac surgery in patients who later developed acute kidney injury. Urinary NGAL has been studied more extensively in paediatric population and has been found to be useful in detecting kidney injury following transplantation, cardiac surgery as well as contrast induced nephropathy early [70, 71].

Other markers that are being studied for their utility in kidney injury are Interleukin-18, Kidney injury molecule 1 (KIM-1), Cystatin – C, Sodium/Hydrogen Exchanger Isoform 3(NHE3) and Liver-type fatty acid binding protein (L-FABP). Kidney injury molecule 1 (KIM-1) and Interleukin-18 are found to be elevated in case of ischemic Acute Tubular Necrosis [72, 73]. Cystatin-C has been found to be better at estimating the GFR than the conventional method using creatinine [74]. Sodium/Hydrogen Exchanger Isoform 3(NHE3), which is found in the urine

following tubular injury, has been found to be better than fractional excretion of sodium in differentiating between pre renal and intrinsic renal causes for renal failure [75]. Liver-type fatty acid binding protein (L-FABP) has also shown promise in animal studies for early detection of AKI and is being adapted as a possible biomarker for AKI [76].

8. Traumatic brain injury

8.1 Traumatic brain injury—acute injury

Traumatic brain injury is a major cause of morbidity and mortality across the world [77]. The reasons are several, but the lion's share of the incidents can be attributed to the high speed motor vehicle collisions. In addition to that, there are other contributing factors like falls as well as injuries due to contact sports. The acute injuries can be devastating and even fatal, but a huge number of those patients also develop chronic neurological sequelae which can be debilitating [78]. Given the complexity of the situation, the understanding of the mechanisms and pathophysiology of these chronic conditions are much less understood compared to their acute counterparts [79].

Traumatic Brain Injury (TBI) can be classified into acute or chronic based on the acuity of the event. Acute traumatic events as a result of MVA (motor vehicle accidents), falls or sporting events mostly result in immediate clinical symptoms or signs that are often diagnosed with the help of neuroimaging immediately by the clinician. A concussion as a result of contact sports or accidents can result in clinical features which can range from mild dizziness to complete unconsciousness. But, it will exhibit no discernible defects in neuroimaging of the patient [80]. Therefore it becomes a clinical diagnosis rather than a radiological diagnosis. Many patients, especially sportspersons who are part of contact sports like boxing have exhibited persistent symptoms for hours to days to months after the insult [81]. The shearing forces in an acceleration/deceleration injury causes axonal damage which is often responsible for the clinical manifestations. The acute event leads to a primary insult to the central nervous system which can manifest as cerebral oedema or even intracranial haemorrhage. This in turn leads to increase in the intracranial pressure which in turn causes cerebral hypoperfusion and resultant tissue hypoxia [82].

Traumatic head injury is not an area which usually requires a biomarker evaluation from the Neurosurgeon's point of view for the purpose of acute care. Clinical decision making is often dependent on neuroimaging, which is a CT scan usually. But, recently, the role of biomarkers has become important in the decision making process of getting a neuroimaging. This is done with a view of reducing the radiation exposure to patients with mild head injury. These biomarkers are not usually present in the circulation and their presence generally indicates a breach of Blood–brain barrier. Some of the important biomarkers described in the recent literature are Glial fibrillary acidic protein (GFAP), calcium binding protein S100B, and tau protein [83]. The most important one in the horizon is S100 calcium binding protein B (S100B), which is a glial-specific protein which is primarily expressed by a subtype of mature astrocytes. It is elevated in neuronal damage which makes it a potential marker for CNS insults. In a study done on 512 adult patients with mild head injury (GCS 14–15, loss of consciousness and/or amnesia and no additional risk factors), the researchers used protein S100B levels as a clinical tool to determine whether the patient needed a CT scan [84]. They found that adult patients with mild head injury, without additional risk factors and with S100B levels of <0.10 mcg/L within 3 hours of injury, can safely be discharged from the hospital without

neuroimaging. A recent study done in Sweden tries to shed light on the utility of biomarkers like total tau, protein S100B and neuron-specific enolase in assessing concussion injuries in sports persons [85].

8.2 Traumatic brain injury - chronic Sequelae

Once the primary insult is over, the brain can suffer from secondary injury as a result of sequelae from the initial insult. This can occur after days, weeks, months or years after the initial event. This results from biochemical cascades that are triggered by the primary event. These secondary events are mediated by free radicals and reactive oxygen species that are generated as a result of tissue hypoxia, reperfusion injury and neuroinflammation [86]. The change in membrane permeability which results from the initial injury causes increase in the calcium uptake or activation of NMDA and AMPA receptors by glutamate can cause mitochondrial dysfunction [87]. Thus the inflammatory response leads to further cellular disruption and the vicious cycle continues to damage the nervous homeostasis. These inflammatory insults in the nervous system give rise to various inflammatory markers which can be detected in the system. These biomarkers have been extensively studied and their usefulness in the clinical environment hotly debated. Even though many of these markers many of them show enough promise within the confines of the laboratory, they are yet to come to the bedside to be used by the clinician in daily practice.

Many animal studies have demonstrated an increase in biochemical markers even after a single day after the insult and these can persist even after a month [88]. Acrolein, a post-traumatic neurotoxin can be quantified in brain tissue and can be elevated depending on the insult. A sustained upregulation has been demonstrated after brain injury, which suggests that it is a potential marker for neuronal injury and inflammation [89].

Proton Magnetic Resonance Spectroscopy (1H-MRS) is a technique which is able to measure the neurochemicals in the nervous system. This helps in detecting the neurotransmitters and metabolites, thereby quantifying the markers in various clinical conditions. Using this method, animal studies have found that endogenous antioxidants glutathione and ascorbic acid may be decreased up to 2 weeks following the insult [90]. F2-isoprostane, a lipid peroxidation by-product, has been found to be elevated on chronic brain injury or Chronic Traumatic Encephalopathy (CTE) which manifests years after the insult(s).

As detected by Proton Magnetic Resonance Spectroscopy (^{1}H-MRS), there have been consistent results in detection of the following neurotransmitters in children with Autism Spectrum Disorder(ASD). There has been reduced levels of N-acetylaspartate (NAA), Creatine and phosphocreatine (Cr + PCr), Glutamine, Myo-Inositol and Choline containing compounds in the subcortical areas as well as cortical white matter and grey matter in varying degrees in children with ASD [91].

8.3 Neurodegenerative diseases

Neurodegenerative diseases can be extremely debilitating and distressing to not only the patient, but also the caregivers. Once diagnosed, the pathophysiological mechanisms can seldom be reversed and hence it becomes the source of social, financial and economic drain for not only families, but for the governmental health machinery itself. The social impact is huge, but the economic impact of these conditions cannot be ignored by any means.

Hence, it becomes imperative that the diagnosis can be made as early as possible thereby mitigating the scenario. An earlier diagnosis will help the clinician as well as the caregivers to come to a plan for further care of the patient. It is also essential

to look at the therapeutic interventions which can arrest or at least slow down the progression of the disease. This is where the role of biomarkers come into picture. A biomarker can help in diagnosing a particular condition early. Even if the diagnosis cannot be confirmed, at least the possibility of the condition can be ascertained and hence be prepared against. This is where a biomarker helps in fighting the diseases which for all practical purposes, have no definitive curative measures available by the bedside.

Biomarkers in neurodegenerative conditions can be classified as fluid and radiological markers. Since radiology is an integral and essential part of assessment of the neurological system in modern medicine, many techniques have been developed which can detect the presence of markers within the brain tissue which can point towards the presence or likelihood of a particular disease condition. On the other hand, there are fluid biomarkers that can be detected in the fluid that flows all over the brain - the cerebrospinal fluid (CSF). Before we look into those markers, it needs to be kept in mind that quite a few markers have gone to the lab in the hunt for that perfect biomarker. But, none of them has been successful enough to be brought to the bedside for daily clinical practice. Among the many neurodegenerative conditions, Alzheimer's disease has been the most extensively studied, because of its prevalence and impact on the society. But the biomarkers used in Alzheimer's disease do overlap with many other conditions as well due to similarities in pathophysiology.

Biomarkers in neurodegenerative conditions can be detected in blood and cerebrospinal fluid (CSF) and are clubbed together to be called as fluid markers. The predominant ones are Amyloid β peptides/oligomers and Tau peptides. Neurofilament light chain (NfL), which is found in myelinated axons, is an important marker which indicates white matter damage and points towards neurodegeneration [92]. Serial NFL sampling in patients at risk of developing Alzheimer's Disease, can be used to predict brain atrophy rates, cognitive impairment and disease progression [93]. These can be estimated by assays in blood as well as CSF. The major disadvantage these markers face is the low specificity and that hinders its utility in clinical practice. Another set of markers or radiological distinction characteristics can be detected in MRI and PET scans and are termed as radiological biomarkers. MRI utilises the various imaging modalities available to detect white matter lesions that are present in many of the neurodegenerative diseases. These include reduction in the volume, thickness, presence of microbleeds, myelin, iron, neuromelanin within the brain tissue in specific regions. PET targets various Tau lesions and Amyloid β aggregates in various regions within the neuronal system to detect the possibility of neurodegeneration. Apart from these, there are genetic biomarkers or specific genes that can predict the possibility of a neurodegenerative disease condition (**Table 4**).

Gene(s)	Disease syndrome associated
APP, PSEN1, PSEN2	Familial - Early-onset Alzheimer's disease
ApoE (ε4 allele), TREM2	AD- Risk factors
MAPT, C9orf72, GRN	Behavioural-variant frontotemporal dementia
VCP, TARDBP, SOD1, FUS	Amyotrophic Lateral Sclerosis
HTT	Huntington's disease
SNCA, GBA	Parkinson's disease
PRNP	Prion Disease

Table 4.
Genetic biomarkers for neurodegenerative diseases.

8.3.1 Alzheimer's disease

It is a proteinopathy which is characterised by accumulation of Tau neurofibrillary tangles and extracellular Amyloid β plaques. These can be assessed by radiological methods as well as by looking at the fluid biomarkers. It has a prolonged pre-clinical phase where there Tau lesions can be found in the subcortical regions even before profound clinical symptoms appear [94]. Similarly amyloid β aggregates are initially found in the neocortical regions and later subcortical and cerebellar regions [95]. Plasma levels of Aβ42 has been found to be decreased compared to controls in a study [96]. The National Institute on Ageing and Alzheimer's Association Research Framework has defined AD by its underlying pathologic processes that can be documented by post-mortem examination or in vivo by biomarkers [97]. The biomarkers are classified into the 3 major groups- β amyloid deposition, pathologic tau, and neurodegeneration - AT(N). As and when newer biomarkers are discovered, they may be added into these categories. When it comes to therapeutics targeted based on these markers, there is still a long way to go to get these implemented in clinical practice. This may be due to the lack of direct correlation between the marker load with the clinical deterioration [98] as well as lack of specificity [99].

8.3.2 Parkinson's disease

It is the most common presentation of synucleinopathy. The presentation can be similar to other similar conditions like Dementia with Lewy bodies. The typical feature can be aggregation of α-synuclein in the form of Lewy bodies which starts in the subcortical regions and later spread into the other regions [100]. Apart from the mutations in the genes, causality has been attributed to pesticide exposure as well as traumatic brain injury [101].

8.3.3 Frontotemporal lobar degeneration

This encompasses a spectrum of disease conditions characterised by vacuolation, gliosis and neuronal loss in the cortical regions of the frontal and temporal lobes. Features of Tau protein accumulation, TDP-43 or fused in sarcoma can be seen in this condition [102]. Since they share a similar pathophysiology, Amyotrophic lateral sclerosis (ALS) and other similar motor neuron diseases are considered to be part of the same spectrum.

Huntington's disease is characterised by progressive neuronal loss and astrogliosis in the striatum along with prominent degeneration of the other cortical regions [103]. Similar to other disease conditions, there is limited data available to look at the therapeutic use of markers in HD also. Plasma levels of IL-8, TNF-α [104], and NfL may become useful in the coming years in this regard. A decrease in the uptake of phosphodiesterase-10 PET tracer in the strial region may become an important marker with regard to therapeutics in HD [105]. Diagnosis of Prion diseases like sporadic Creutzfeldt-Jakob disease (sCJD) is done by EEG, MRI or CSF based biomarkers with the use of real-time quaking-induced conversion (RT-QuIC) which is preferred over 14-3-3 protein detection [106].

Since many of the neurodegenerative conditions present late in clinical practice, it is imperative that in order to tackle this menace, the scientific community will have to bring forth tools that can detect these early as well us much ahead of the phase of clinical presentation, even decades. It is in this regard, the biomarkers have a major role to play, whether they are radiological or fluid markers. Genetic markers are useful when we are dealing with hereditary conditions or familial variants of

neurodegenerative conditions. Confirmation of the diagnosis is essential in determining the treatment and initiating it at the earliest for the best possible response.

8.4 Newer modalities in the horizon

Even though the field of biomarker evaluation is not very old, it is a fast changing world and newer techniques are being added to the mix quite often. A recent technique is the Multimer Detection System-Oligomeric Aβ, which looks at the tendency of plasma proteins to oligomerize [107]. Immune-infrared sensor assay to measure blood vessels for the propensity of the amyloid protein to form β-sheets has also been tried with some success as a potential biomarker [108]. Measurement of locus coeruleus, an early affected region, using special MRI techniques is also being explored as a potential target [109].

9. Point of care tests (POCT)

POCT refers to diagnostic evaluation at or near the site of patient care. A POC lab is not within the institutional central laboratory, but nearer to the patient care setting like ED or ICU. POC testing of biomarkers is increasingly becoming the norm at the moment. This has been touted as the next revolutionary step in faster healthcare delivery in the Emergency department. But, the challenge lies in transferring the resultant advantage to improvement in patient care and disposition. The benefit demonstrated on paper should be translated to better patient care by the bedside. If the results of the POC testing do not alter the course of management of the patient, it defeats the whole purpose of POCT.

10. Future of biomarker use in emergency medicine

Biomarkers are being increasingly used in the Emergency departments for faster patient disposition. Recently efforts are being undertaken to include an array of biomarkers in the triage scoring itself as a method of risk stratification of patients presenting to the ED [110]. In this study, the researchers included biomarkers from 3 distinct biological pathways for risk stratification of general medical patients presenting to the ED. The study included biomarkers of inflammation (pro-adrenomedullin [ProADM]), stress (copeptin) and infection (procalcitonin). They used a multi-marker approach to stratify patients and came to a conclusion that all the markers strongly predicted the risk of death, ICU admission and high initial triage priority, especially ProADM. It is a possibility that these methods may get introduced in clinical practice in the not so distant future.

11. Conclusion

Biomarkers are among the best tools in the hand of the clinicians at present. Each and every clinical condition has been tagged with a quantifiable biomarker which helps in faster clinical diagnosis as well as prognostication. Overall this would lead towards better healthcare delivery to the patient. But, the sheer vast numbers and volumes of various biomarkers in the research pipeline points towards a glaring fact. There is no single marker that can give a complete picture of the patient's clinical condition. There is no ideal biomarker. A scoring system based on multiple

markers would give a better picture than a single one. This accuracy always comes at a higher cost, which translates to more expensive healthcare delivery. There is dire requirement for better clinical validation among the various contenders in each disease process A biomarker based evaluation system, though more accurate, may not suit each and every healthcare facility, but needs to be tailored based on the adaptability and cost effectiveness suited to the society it caters to. Given the vast array of biomarker assays, clinicians should keep in mind that these should always be used as tools that compliment your clinical decision making process rather than replacing the process itself.

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Conflict of interest

I have no conflict of interest to declare.

Abbreviations

ACC	American College of Cardiology
ACS	Acute Coronary Syndrome
AST	Aspartate aminotransferase
BNP	B type natriuretic peptide
CABG	Coronary Artery Bypass Grafting
CHF	Congestive Heart Failure
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CT	Computed Tomography
ECG	Electrocardiogram
ED	Emergency Department
H-FABP	Heart Fatty Acid Binding Protein
ICU	Intensive Care Unit
IL	Interleukin
IMA	Ischemia modified albumin
LDH	Lactate dehydrogenase
LVF	Left Ventricular Failure
MI	Myocardial Infarction
MR-proADM	Mid-regional Proadrenomedullin
NACB	National Academy of Clinical Biochemistry
NGAL	Neutrophil gelatinase-associated lipocalin
NSTEMI	Non ST Elevation Myocardial Infarction
NT pro BNP	N-Terminal pro B type natriuretic peptide
PCI	Percutaneous Coronary Intervention
POCT	Point of Care Testing
QC	Quality Control

STEMI	ST Elevation Myocardial Infarction
TNF	Tumour Necrosis Factor
TNF-alpha	Tumour necrosis factor-alpha

Author details

Anoop T. Chakrapani
Frimley Park Hospital, Frimley, United Kingdom

*Address all correspondence to: dranooptc@gmail.com

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References

[1] Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clinical Pharmacology and Therapeutics. 2001;**69**(3):89-95

[2] Chakrapani AT. Biomarkers in emergency medicine. In: David S, editor. Clinical Pathways in Emergency Medicine. New Delhi: Springer; 2016. DOI: https://doi.org/10.1007/978-81-322-2710-6_32

[3] Schuetz P, Aujesky D, Muller C, Muller B. Biomarker-guided personalised emergency medicine for all - hope for another hype? Swiss Medical Weekly. 2015;**145**:w14079

[4] Hollander JE, Than M, Mueller C. State-of-the-art evaluation of emergency department patients presenting with potential acute coronary syndromes. Circulation. *2016*;**134**:547-564. DOI: 10.1161/CirculationAHA.116.021886

[5] Saenger AK, Beyrau R, Braun S, Cooray R, Dolci A, Freidank H, et al. Multicentre analytical evaluation of a high-sensitivity troponin T assay. Clinica Chimica Acta. 2011 Apr 11;**412**(9-10):748-754

[6] Morrow DA, Cannon CP, Jesse RL, et al. National Academy of Clinical Biochemistry Laboratory medicine practice guidelines: Clinical characteristics and utilization of biochemical markers in acute coronary syndromes. Clinical Chemistry. 2007;**53**:552-574

[7] Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-Elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. Aug 14 2007;**50**(7)

[8] Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, Caso P, Dudek D, Gielen S, Huber K, Ohman M, Petrie MC, Sonntag F, Uva MS, Storey RF, Wijns W, Zahger D: ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC).ESC Committee for Practice Guidelines. Eur Heart J. 2011 Dec; 32(23):2999-3054.

[9] Diercks D.B, Peacock W.F, Hollander J.E, Singer A.J, Birkhahn R, Shapiro N: Diagnostic accuracy of a point-of-care troponin I assay for acute myocardial infarction within 3 hours after presentation in early presenters to the emergency department with chest pain. Am Heart J 2012;163:74-80.e4

[10] Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Journal of the American College of Cardiology. 2012;**60**:1581-1598

[11] Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. N Engl J Med. Oct 19 2000;343(16):1139-1147

[12] Newby LK, Christenson RH, OhmanEM,ArmstrongPW,ThompsonTD, Lee KL, et al. Value of serial troponin T measures for early and late risk stratification in patients with acute coronary syndromes. The GUSTO-IIa

Investigators. Circulation. Nov 3 1998;98(18):1853-1859

[13] Newby et al. ACCF 2012 Expert Consensus Document on Practical Clinical Considerations in the Interpretation of Troponin Elevations, A Report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. JACC Vol. 60, No. 23, December 11, 2012:2427-2463

[14] De Gennaro GL, Brunetti ND, Cuculo A, et al. Increased troponin levels in nonischemic cardiac conditions and noncardiac diseases. Journal of Interventional Cardiology. 2008;**21**:129-139

[15] Diamond GA, Kaul S. How would the reverend Bayes interpret high-sensitivity troponin? Circulation. 2010;**121**:1172-1175

[16] Eggers KM, Oldgren J, Nordenskjöld A, Lindahl B. Diagnostic value of serial measurement of cardiac markers in patients with chest pain: Limited value of adding myoglobin to troponin I for exclusion of myocardial infarction. American Heart Journal. Oct 2004;**148**(4):574-581

[17] Conor J. McCann Ben M. Glover Ian B.A. Menown Michael J. Moore Jane McEneny Colum G. Owens Bernie Smith Peter C. Sharpe Ian S. Young Jennifer A. Adgey; Novel biomarkers in early diagnosis of acute myocardial infarction compared with cardiac troponin T European Heart Journal, Volume 29, Issue 23, 1 December 2008, Pages 2843-2850,https://doi.org/10.1093/eurheartj/ehn363

[18] Maisel AS, Clopton P, Krishnaswamy P, Novak RM, McCord J, Hollander JE, et al. Impact of age, race, and sex on the ability of B-type natriuretic peptide to aid in the emergency diagnosis of heart failure: Results from the breathing not properly (BNP) multinational study. American Heart Journal. 2004;**147**(6):1078-1084

[19] Kim HN, Januzzi JL Jr. Natriuretic peptide testing in heart failure. Circulation. 2011;**123**:2015-2019

[20] Ray P, Delerme S, Jourdain P, Chenevier-Gobeaux C. Differential diagnosis of acute dyspnoea: the value of B natriuretic peptides in the emergency department. QJM. 2008;**101**:831-843

[21] Savarese G, Trimarco B, Dellegrottaglie S, Prastaro M, Gambardella F, Rengo G, et al. Natriuretic peptide-guided therapy in chronic Heart Failure: A meta-analysis of 2,686 patients in 12 randomized trials. PLoS One. 2013;**12**:e58287

[22] Marcondes-Braga FG, Gutz IGR, Batista GL, Saldiva PHN, Ayub-Ferreira SM, Issa VS, et al. Exhaled acetone as a new biomarker of heart failure severity. Chest. 2012 Aug;**142**(2):457-466

[23] Ky B, French B, Levy WC, et al. Multiple biomarkers for risk prediction in chronic heart failure. Circulation. Heart Failure. 2012;**5**:183-190

[24] Tang WH, Francis GS, Morrow DA, et al. National academy of clinical biochemistry laboratory medicine practice guidelines: Clinical utilization of cardiac biomarker testing in heart failure. Circulation. 2007;**116**:e99-e109

[25] De Boer RA, Yu L, van Veldhuisen DJ. Galectin-3 in cardiac remodelling and heart failure. Current Heart Failure Reports. 2010;**7**:1-8

[26] Maisel A, Mueller C, Nowak R, Peacock WF, Landsberg JW, Ponikowski P, Mockel M, Hogan C, Wu AH, Richards M, Clopton P, Filippatos GS, Di Somma S, Anand I, Ng L, Daniels LB, Neath SX, Christenson R, Potocki M, McCord J, Terracciano G, Kremastinos D, Hartmann O, von Haehling S,

Bergmann A, Morgenthaler NG, Anker SD. Mid-Region pro-hormone markers for diagnosis and prognosis in acute dyspnoea: results from the BACH (Biomarkers in acute heart failure) Trial, J Am Coll Cardiol , 2010, vol. 55 (pg. 2062-2076)

[27] Kearon C, Ginsberg JS, Douketis J, Turpie AG, Bates SM, Lee AY, et al. An evaluation of D-dimer in the diagnosis of pulmonary embolism: a randomized trial. Ann Intern Med. Jun 6 2006;144(11):812-821.

[28] Geersing GJ, Erkens PM, Lucassen WA, Büller HR, Cate HT, Hoes AW, et al. Safe exclusion of pulmonary embolism using the Wells rule and qualitative D-dimer testing in primary care: prospective cohort study. BMJ. Oct 4 2012;345:e6564

[29] Turedi S, Gunduz A, Mentese A, Topbas M, Karahan SC, Yeniocak S, et al. The value of ischemia-modified albumin compared with d-dimer in the diagnosis of pulmonary embolism. Respir Res. May 30 2008;9:49.

[30] Klok FA, Mos IC, Huisman MV. Brain-type natriuretic peptide levels in the prediction of adverse outcome in patients with pulmonary embolism: a systematic review and meta-analysis. Am J Respir Crit Care Med. Aug 15 2008;178(4):425-430

[31] Levy MM, Dellinger RP, Townsend SR, et al. The surviving sepsis campaign: results of an international guideline-based performance improvement program targeting severe sepsis. Intensive Care Medicine. 2010;**36**:222-231

[32] Pierrakos C, Vincent JL. Sepsis biomarkers: A review. Critical Care. 2010;**14**(1):R15

[33] Vincent JL, Donadello K, Schmit X. Biomarkers in the critically ill patient: C-reactive protein. Critical Care Clinics. 2011;**27**:241-251

[34] Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: A systematic review and meta-analysis. Clinical Infectious Diseases. 2004;**39**:206-217

[35] Cho SY, Choi JH; Biomarkers of Sepsis; Infect Chemother. Mar 2014; 46(1): 1-12

[36] Póvoa P, Coelho L, Almeida E, Fernandes A, Mealha R, Moreira P, et al. C-reactive protein as a marker of infection in critically ill patients. Clinical Microbiology and Infection. 2005;**11**:101-108

[37] Becker KL, Nylen ES, White JC, Muller B, Snider RHJ. Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: A journey from calcitonin back to its precursors. The Journal of Clinical Endocrinology and Metabolism. 2004;**89**(4):1512-1525

[38] Long B, Koyfman A. Ready for prime time? Biomarkers in sepsis. Emergency Medicine Clinics of North America. 2017;**35**(1):109-122

[39] Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: A systematic review and meta-analysis. Clinical Infectious Diseases. 2004;**39**:206-217

[40] Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2013;**13**:426-435

[41] Kopterides P, Siempos II, Tsangaris I, Tsantes A, Armaganidis A. Procalcitonin-guided algorithms of antibiotic therapy in the intensive care unit: A systematic review

and meta-analysis of randomized controlled trials. Critical Care Medicine. 2010;**38**:2229-2241

[42] Mikkelsen ME, Miltiades AN, Gaieski DF, et al. Serum lactate is associated with mortality in severe sepsis independent of organ failure or shock. Critical Care Medicine. 2009;**37**:1670-1677

[43] Shapiro NI, Howell MD, Talmor D, Nathanson LA, Lisbon A, Wolfe RE, et al. Serum lactate as a predictor of mortality in emergency department patients with infection. Annals of Emergency Medicine. 2005;**45**:524-528

[44] Nguyen HB, Rivers EP, Knoblich BP, Jacobsen G, Muzzin A, Ressler JA, et al. Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. Critical Care Medicine. 2004;**32**:1637-1642

[45] Lewis LK, Smith MW, Yandle TG, Richards AM, Nicholls MG. Adrenomedullin(1-52) measured in human plasma by radioimmunoassay: Plasma concentration, adsorption, and storage. Clinical Chemistry. 1998;**44**(3):571-577

[46] Maisel A, Mueller C, Nowak R, Peacock WF, Landsberg JW, Ponikowski P, et al. Mid-region pro-hormone markers for diagnosis and prognosis in acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. J Am Coll Cardiol. 2010 May 11;**55**(19):2062-2076

[47] Gille J, Ostermann H, Dragu A, Sablotzki A. MR-proADM: A new biomarker for early diagnosis of sepsis in burned patients. Journal of Burn Care & Research. 2017 Mar;**28**

[48] Gijs W.D. Landman, Peter R. van Dijk, Iefke Drion, Kornelis J.J. van Hateren, Joachim struck, Klaas H. Groenier, Rijk O.B. Gans, Henk J.G. Bilo, Stephan J.L. Bakker, NanneKleefstra; Midregional fragment of Proadrenomedullin, new-onset albuminuria, and cardiovascular and all-cause mortality in patients with type 2 diabetes (ZODIAC-30); Diabetes Care Mar 2014, 37 (3) 839-845

[49] Oberholzer A, Souza SM, Tschoeke SK, Oberholzer C, Abouhamze A, Pribble JP, et al. Plasma cytokine measurements augment prognostic scores as indicators of outcome in patients with severe sepsis. Shock. 2005 Jun;**23**(6):488-493

[50] Sundén-Cullberg J, Norrby-Teglund A, Rouhiainen A, Rauvala H, Herman G, Tracey KJ, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Critical Care Medicine. 2005 Mar;**33**(3):564-573

[51] Bozza FA, Gomes RN, Japiassú AM, Soares M, Castro-Faria-Neto HC, Bozza PT, et al. Macrophage migration inhibitory factor levels correlate with fatal outcome in sepsis. Shock. 2004;**22**:309-313

[52] Gaïni S, Koldkjaer OG, Pedersen C, Pedersen SS. Procalcitonin, lipopolysaccharide-binding protein, interleukin-6 and C-reactive protein in community-acquired infections and sepsis: A prospective study. Critical Care. 2006;**10**(2):R53

[53] Sakr Y, Burgett U, Nacul FE, Reinhart K, Brunkhorst F. Lipopolysaccharide binding protein in a surgical intensive care unit: A marker of sepsis? Critical Care Medicine. 2008 Jul;**36**(7):2014-2022

[54] Nunez Lopez O, Cambiaso-Daniel J, Branski LK, Norbury WB, Herndon DN. Predicting and managing sepsis in burn patients: Current perspectives. Therapeutics and Clinical Risk Management. 2017;**13**:1107-1117

[55] Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High

serum procalcitonin concentrations in patients with sepsis and infection. Lancet (London, England) 1993;341(8844):515-518

[56] Cabral L, Afreixo V, Almeida L, Paiva JA. The use of procalcitonin (PCT) for diagnosis of sepsis in burn patients: A meta-analysis. PLoS One. 2016;**11**(12):e0168475

[57] Parameswaran N, Patial S. Tumor necrosis factor-alpha signalling in macrophages. Critical Reviews in Eukaryotic Gene Expression. 2010;**20**(2):87-103

[58] Abdel-Hafez NM, Saleh Hassan Y, El-Metwally TH. A study on biomarkers, cytokines, and growth factors in children with burn injuries. Annals of Burns and Fire Disasters. 2007;**20**(2):89-100

[59] Arslan E, Yavuz M, Dalay C. The relationship between tumor necrosis factor (TNF)-alpha and survival following granulocyte-colony stimulating factor (G-CSF) administration in burn sepsis. Burns. 2000;**26**(6):521-524

[60] Finnerty CC, Herndon DN, Przkora R, et al. Cytokine expression profile over time in severely burned pediatric patients. Shock. 2006;**26**(1):13-19

[61] Carsin H, Assicot M, Feger F, et al. Evolution and significance of circulating procalcitonin levels compared with IL-6, TNF alpha and endotoxin levels early after thermal injury. Burns. 1997;**23**(3):218-224

[62] Ma L, Zhang H, Yin Y, et al. Role of interleukin-6 to differentiate sepsis from non-infectious systemic inflammatory response syndrome. Cytokine. 2016;**88**:126-135

[63] Kraft R, Herndon DN, Finnerty CC, Cox RA, Song J, Jeschke MG. Predictive value of IL-8 for sepsis and severe infections after burn injury: A clinical study. Shock. 2015;**43**(3):222-227

[64] Csontos C, Foldi V, Palinkas L, et al. Time course of pro- and anti-inflammatory cytokine levels in patients with burns – Prognostic value of interleukin-10. Burns. 2010;**36**(4):483-494

[65] Cakir Madenci O, Yakupoglu S, Benzonana N, Yucel N, Akbaba D, Orcun KA. Evaluation of soluble CD14 subtype (presepsin) in burn sepsis. Burns. 2014;**40**(4):664-669

[66] Zhang J, Hu ZD, Song J, et al. Diagnostic value of Presepsin for sepsis: A systematic review and meta-analysis. Medicine (Baltimore). 2015;**94**:e2158

[67] Barber R, Aragaki C, Rivera-Chavez F, Purdue G, Hunt J, Horton J. TLR4 and TNF-α polymorphisms are associated with an increased risk for severe sepsis following burn injury. Journal of Medical Genetics. 2004;**41**(11):808-813

[68] Bauer M, Giamarellos-Bourboulis EJ, Kortgen A, et al. A transcriptomic biomarker to quantify systemic inflammation in sepsis – A prospective multicentre phase II diagnostic study. eBioMedicine. 2016;**6**:114-125

[69] Mishra J, Mori K, Ma Q, Kelly C, Yang J, Mitsnefes M, et al. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. Dec 2004;**15**(12):3073-3082

[70] Mishra J, Ma Q, Kelly C, Mitsnefes M, Mori K, Barasch J, et al. Kidney NGAL is a novel early marker of acute injury following transplantation. Pediatric Nephrology. Jun 2006;**21**(6):856-863

[71] Hirsch R, Dent C, Pfriem H, Allen J, Beekman RH 3rd, Ma Q, et al.

NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatric Nephrology. Dec 2007;**22**(12):2089-2095

[72] Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney injury molecule-1 (KIM-1): A novel biomarker for human renal proximal tubule injury. Kidney International. Jul 2002;**62**(1):237-244

[73] Parikh CR, Jani A, Melnikov VY, Faubel S, Edelstein CL. Urinary interleukin-18 is a marker of human acute tubular necrosis. American Journal of Kidney Diseases. Mar 2004;**43**(3):405-414

[74] Waheed S, Matsushita K, Sang Y, et al. Combined association of albuminuria and cystatin C-based estimated GFR with mortality, coronary heart disease, and heart failure outcomes: the atherosclerosis risk in communities (ARIC) study. American Journal of Kidney Diseases. 2012;**60**:207-216

[75] du Cheyron D, Daubin C, Poggioli J, Ramakers M, Houillier P, Charbonneau P, et al. Urinary measurement of Na+/H+ exchanger isoform 3 (NHE3) protein as new marker of tubule injury in critically ill patients with ARF. American Journal of Kidney Diseases. Sep 2003;**42**(3):497-506

[76] Doi K, Noiri E, Sugaya T. Urinary L-type fatty acid-binding protein as a new renal biomarker in critical care. Current Opinion in Critical Care. 2010 Dec;**16**(6):545-549

[77] Ghajar J. Traumatic brain injury. Lancet. 2000;**356**:923-929

[78] Riggio S, Wong M. Neurobehavioral sequelae of traumatic brain injury. Mt. Sinai J. Med. J. Transl. Pers. Med. 2009;**76**:163-172

[79] Johnson VE, Stewart W, Smith DH. Widespread tau and amyloid-Beta pathology many years after a single traumatic brain injury in humans: Long-term AD-like pathology after single TBI. Brain Pathology. 2012;**22**:142-149

[80] McCrory P, Meeuwisse W, Johnston K, Dvorak J, Aubry M, Molloy M, et al. consensus statement on concussion in sport - the third international conference on concussion in sport held in Zurich, November 2008;Phys. Sportsmed. 2009;**37**:141-159

[81] Sterr A, Herron KA, Hayward C, et al. Are mild head injuries as mild as we think? Neurobehavioral concomitants of chronic post-concussion syndrome. BMC Neurology. 2006;**6**:7 https://doi.org/10.1186/1471-2377-6-7

[82] Bratton SL, Chestnut RM, Ghajar J, McConnell Hammond FF, Harris OA, Hartl R, et al. Guidelines for the management of severe traumatic brain injury. VI. Indications for intracranial pressure monitoring. Journal of Neurotrauma. 2007;**24** (Suppl 1):S37-S44

[83] Kulbe JR, Geddes JW. Current status of fluid biomarkers in mild traumatic brain injury. Experimental Neurology. 2016;**275**:334-352

[84] Calcagnile O, Unden L, Unden J. Clinical validation of S100B use in management of mild head injury. BMC Emergency Medicine. 2012;**12**:13

[85] Bazarian JJ, Merchant-Borna K. Tau, s-100 calcium-binding protein B, and neuron-specific enolase as biomarkers of concussion. JAMA Neurology. 2014 Jul 1;**71**(7):925-926

[86] Bang SA, Song YS, Moon BS, Lee BC, Lee H, Kim J-M, et al. Neuropsychological, metabolic, and GABA A receptor studies in subjects with repetitive traumatic brain injury. Journal of Neurotrauma. 2016;**33**:1005-1014

[87] Zhang Y, Bhavnani BR. Glutamate-induced apoptosis in neuronal cells is mediated via caspase-dependent and independent mechanisms involving calpain and caspase-3 proteases as well as apoptosis inducing factor (AIF) and this process is inhibited by equine oestrogens. BMC Neuroscience. 2006;7:49

[88] Ahmed FA, Kamnaksh A, Kovesdi E, Long JB, Agoston DV. Long-term consequences of single and multiple mild blast exposure on select physiological parameters and blood-based biomarkers. Electrophoresis. 2013;**34**:2229-2233

[89] Walls MK, Race N, Zheng L, Vega-Alvarez SM, Acosta G, Park J, et al. Structural and biochemical abnormalities in the absence of acute deficits in mild primary blast-induced head trauma. Journal of Neurosurgery JNS. 2016;**124**(3):675-686

[90] Harris JL, Yeh H-W, Choi I-Y, Lee P, Berman NE, Swerdlow RH, et al. Altered neurochemical profile after traumatic brain injury: 1H-MRS biomarkers of pathological mechanisms. Journal of Cerebral Blood Flow and Metabolism. 2012;**32**:2122-2134

[91] A Comprehensive Review of the 1H-MRS Metabolite Spectrum in Autism Spectrum Disorder; T.Ford,D. Crewther; Frontiers in Molecular Neuroscience;2016

[92] Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. Lewczuk P, Ermann N, Andreasson U, Schultheis C, Podhorna J, Spitzer P, Maler JM, Kornhuber J, Blennow K, Zetterberg H. Alzheimer's Research & Therapy 2018 Jul 28; 10(1):71.

[93] Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Preische O, Schultz SA , Apel A , et al. Nature Medicine 2019;25:277-283.

[94] NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R; Alzheimer's & Dementia 2018 Apr; 14(4):535-562.

[95] Phases of A beta-deposition in the human brain and its relevance for the development of AD. Thal DR, Rüb U, Orantes M, Braak H; Neurology 2002 Jun 25; 58(12):1791-1800.

[96] High performance plasma amyloid β biomarkers for Alzheimer's disease. *Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, Fowler C, Li QX, Martins R, Rowe C, Tomita T, Matsuzaki K, Ishii K, Ishii K, Arahata Y, Iwamoto S, Ito K, Tanaka K, Masters CL, Yanagisawa K. Nature. 2018 Feb 8; 554(7691):249-254.*

[97] NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R. Alzheimer's & Dementia 2018 Apr; 14(4):535-562

[98] Persistent neuropathological effects 14 years following amyloid β immunization in Alzheimer's disease. *Nicoll JAR, Buckland GR, Harrison CH, Page A, Harris S, Love S, Neal JW, Holmes C, Boche D.Brain. 2019 Jul 1; 142(7):2113-2126.*

[99] Association of Plasma Neurofilament Light With Neurodegeneration in Patients With

Alzheimer Disease. *Mattsson N, Andreasson U, Zetterberg H, Blennow K, Alzheimer's Disease Neuroimaging Initiative. JAMA Neurol. 2017 May 1; 74(5):557-566.*

[100] Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. Cell and Tissue Research. 2004;**318**(1):121-134. DOI: 10.1007/s00441-004-0956-9

[101] Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Annals of Neurology. 2012;**72**(6):893-901. DOI: 10.1002/ana.23687

[102] Mackenzie IR, Neumann M, Cairns NJ, Munoz DG, Isaacs AM. Novel types of frontotemporal lobar degeneration: Beyond tau and TDP-43. Journal of Molecular Neuroscience. 2011;**45**(3):402-408. DOI: 10.1007/s12031-011-9551-1

[103] Rub U, Seidel K, Heinsen H, Vonsattel JP, den Dunnen WF, Korf HW. Huntington's disease (HD): the neuropathology of a multisystem neurodegenerative disorder of the human brain. Brain Pathology. 2016;**26**(6):726-740. DOI: 10.1111/bpa.12426

[104] A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. Björkqvist M, Wild EJ, Thiele J, Silvestroni A, Andre R, Lahiri N, Raibon E, Lee RV, Benn CL, Soulet D, Magnusson A, Woodman B, Landles C, Pouladi MA, Hayden MR, Khalili-Shirazi A, Lowdell MW, Brundin P, Bates GP, Leavitt BR, Möller T, Tabrizi SJ; J Exp Med. 2008 Aug 4; 205(8):1869-1877.

[105] The phosphodiesterase 10 positron emission tomography tracer, [18F] MNI-659, as a novel biomarker for early Huntington disease. *Russell DS, Barret O, Jennings DL, Friedman JH, Tamagnan GD, Thomae D, Alagille D, Morley TJ, Papin C, Papapetropoulos S, Waterhouse RN, Seibyl JP, Marek KL. JAMA Neurol. 2014 Dec; 71(12):1520-1528.*

[106] Accuracy of diagnosis criteria in patients with suspected diagnosis of sporadic Creutzfeldt-Jakob disease and detection of 14-3-3 protein, France, 1992 to 2009. *Peckeu L, Delasnerie-Lauprètre N, Brandel JP, Salomon D, Sazdovitch V, Laplanche JL, Duyckaerts C, Seilhean D, Haïk S, Hauw JJ. Euro Surveill. 2017* Oct*; 22(41)*

[107] Dynamic changes of oligomeric amyloid β levels in plasma induced by spiked synthetic Aβ42. *An SSA, Lee BS, Yu JS, Lim K, Kim GJ, Lee R, Kim S, Kang S, Park YH, Wang MJ, Yang YS, Youn YC, Kim S; Alzheimers Res Ther. 2017 Oct 17; 9(1):86*

[108] Amyloid β-Secondary Structure Distribution in Cerebrospinal Fluid and Blood Measured by an Immuno-Infrared-Sensor: A biomarker candidate for Alzheimer's Disease.*Nabers A, Ollesch J, Schartner J, Kötting C, Genius J, Hafermann H, Klafki H, Gerwert K, Wiltfang J. Anal Chem. 2016* Mar 1*; 88(5):2755-2762*

[109] Locus coeruleus imaging as a biomarker for noradrenergic dysfunction in neurodegenerative diseases. *Betts MJ, Kirilina E, Otaduy MCG, Ivanov D, Acosta-Cabronero J, Callaghan MF et al. Brain. 2019 Sep 1; 142(9):2558-2571*

[110] Schuetz P, Hausfater P, Amin D, et al. Biomarkers from distinct biological pathways improve early risk stratification in medical emergency patients: the multinational, prospective, observational TRIAGE study. Critical Care. 2015;**19**:377