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# Biomarkers and Physiological Agents in Severe Sepsis and Septic Shock

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## 1. Introduction

The term sepsis implies the presence of infection with signs of systemic body response (tachycardia, tachypnea, fever, etc.) and conditions of severe sepsis and septic shock, further disturbance in organ perfusion (impaired consciousness, hypoxia, oliguria) required fluid administration and inotropic and / or vasopressor drugs, respectively (Levy et al. 2003, Bone et al.1992).

Sepsis is an enormously complex clinical syndrome that arises from the activation of an innate host response to danger. Sepsis is associated with infections of bacteria, viruses, fungi and endotoxins, whether or not being evidenced by culture. The above systematic inflammatory response in humans occurs not only in sepsis but also in other non-infectious conditions such as pancreatitis, ischemia, severe trauma, etc. The host responds in the same way in infectious as in non-infectious events, which is described by the term systemic inflammatory response syndrome (SIRS). Insights into the biology of innate immunity suggest that in terms of impact on outcomes, the specific insult evoking the response is less important than the nature of the response itself. The terms sepsis and septicemia were- until relatively recently- used indiscriminately. However, researchers tend to use those two terms with different and distinct meanings according to several conditions under which each of this condition happens (Pezilli et al, 2010, Dhar et al, 2008, Hoover et al, 2006, Matzinger et al, 2002, Bone et al, 1992). Sepsis is a very complex chain of events including inflammatory and anti-inflammatory processes, circulatory abnormalities and humoral and cellular reactions. Its complexity, in addition to high variability of its non-specific signs and symptoms, make early diagnosis and determination of its severity very important, as sepsis can be lethal for patients. Early diagnosis increases the possibility of starting a specific therapy in time (Lever et al, 2007, Hotchkiss and Karl, 2003, Zambon et al., 2008).

As mentioned above, sepsis may be initialized by local inflammation in order to inactivate invading factors. At first the host recruits and activates leukocytes at infectious foci. During that phase, innate immune cells recognize invading factors through pattern recognition receptors (PRRs) on the cells. Toll-like receptors (TLRs), which belong to PRRs, activate

immune cells. This activation is caused by the production of pro-inflammatory cytokines and chemokines. Bacterial products and viral proteins are linked to various and distinct TLRs and are the main cause of cytokine and chemokine production. Following the complement is activated which activates leukocytes. Cells activated through all this process produce and secrete many agents such as leukotriene, nitric oxide and inducible nitric oxide synthase (iNOS) and free radicals. All this process is initiated by many proinflammatory mediators (cytokines, adhesion molecules, vasodilating mediators and reactive oxygen species). If the above body reaction fails to achieve its goals, then some of the invading factors may invade into the bloodstream and trigger a systemic inflammatory reaction. This happens mainly through overproduction of inflammatory cytokines (eg TNF, IFNs and IL-1,6). In all the inflammatory process many agents are overproduced and are of high importance for patients health. Apoptosis has a key role in sepsis and it is influenced by many factors such as caspases, pro-inflammatory cytokines release –such as TNF-a, IFN-γ, IL-1β - and anti-inflammatory cytokines e.g. TGFβ, IL-10 and IL-13, many different pathogenic toxins, such as LPS, CLP and CASP, while it happens in many organs such as heart, glomeruli, liver, bone marrow, thymus, spleen, lungs and gut. The TNFR- CD40-TRADD- FADD pathway –activated by TNF-a- leads to inhibition of cas-8, which is an initiator factor of apoptosis, and therefore that pathway inhibits apoptosis. Additionally, neutrophile and monocyte activation leads to inhibition of Bfl-1, which belongs to Bcl-2 family and inhibits apoptosis. On the contrary, activation of Cardinal and Fas death receptor by Bim (of the Bcl-2 family), which leads to activation of lymphocyte apoptosis, causes a decline of inflammatory response. Apoptosis inhibition leads to SIRS, while its activation leads to immunoparalysis and MODS. However, apoptosis inhibition may have a beneficial role for septic patient. Immunodeficiency is another characteristic of sepsis, which happens due to increased apoptosis of CD4 T cells, dendritic cells and B cells and leads to immunoparalysis. The coagulation system is activated by cytokines and oxidants through tissue factor induction. Antithrombin III plays a dominant role among antithrombotic and fibrinolytic agents. Protein C or autoprothrombin IIA, protect cells from sepsis and it has strong anticoagulative effects through inactivating Va and VIIIa factors of the coagulation system (Remick, 2007, Okazaki and Matsukawa, 2009, Hotchkiss et al, 2003, McDonald, et al 2000, Sohn et al 2003, Hildeman et al 2002, O'Neill et al 2000). (**Figure 1**)

Physiological agents participating in sepsis pathophysiology cascades are used as biomarkers in sepsis diagnosis. Biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic process or pharmacologic responses to a therapeutic intervention (BDWG, 2001). The exact role of biomarkers in septic patient's management is not yet defined. Nevertheless, biomarkers can play an important role in early diagnosis and severity determination of sepsis. Moreover they can differentiate bacterial from viral and fungal infection and systemic from local infection. Differentiation of Gram-positive from Gram-negative microorganisms as cause of sepsis is a potential use of biomarkers. Biomarkers may also be used in guiding antibiotic therapy, guiding therapy, and evaluating the response to therapy. Other possible biomarker applications are prediction of sepsis complications and development of organ dysfunction (BDWG, 2001, Marshall and Reinhart, 2009, Dellinger et al, 2008). It has been shown that various biomarkers are very useful in clinical practice in sepsis diagnosis and some of them are thought superior to clinical signs as far as diagnosis.

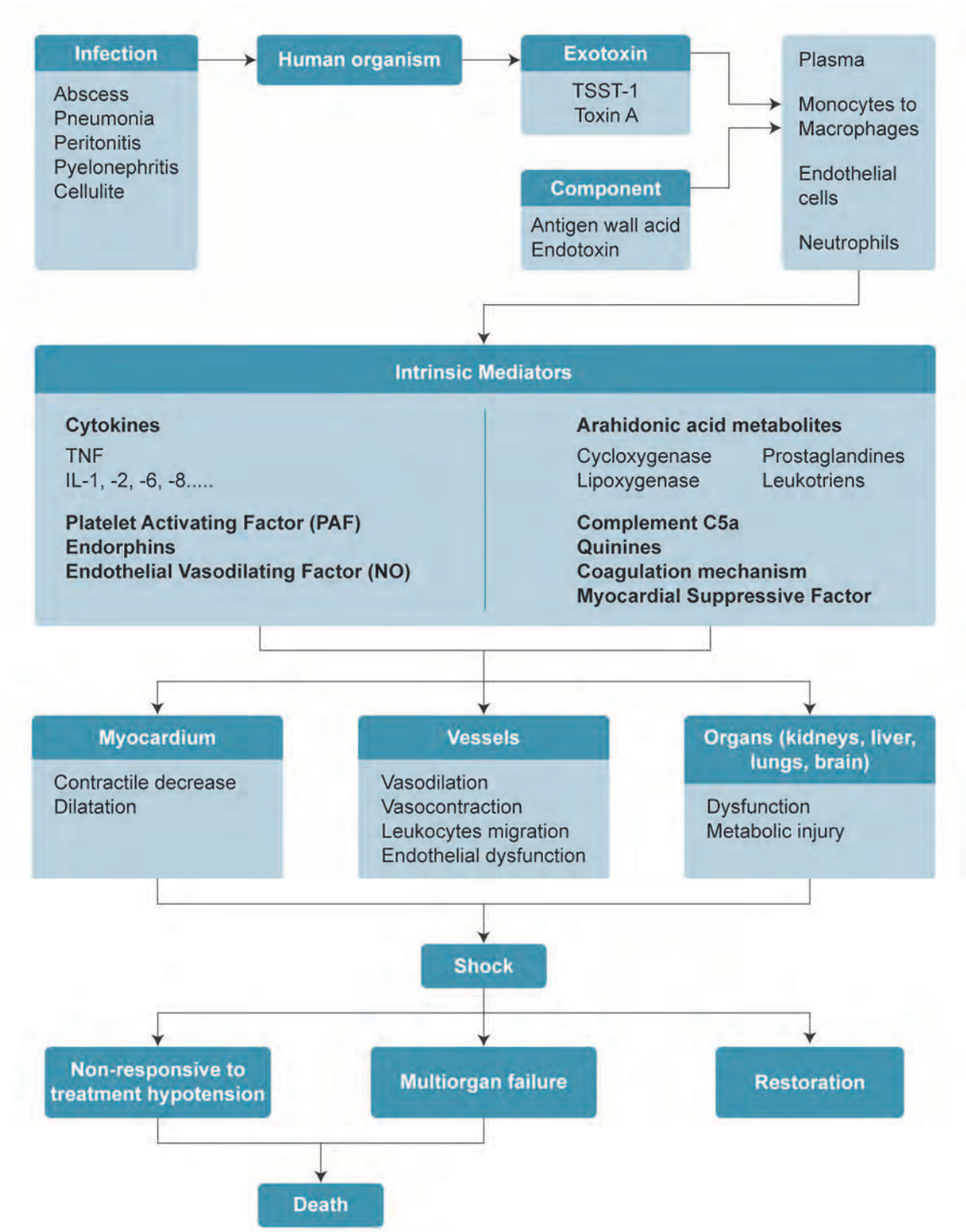


Fig. 1. Pathogenetic mechanism of sepsis (improved figure by Parillo et al, 1993)

Biomarkers of sepsis can be divided in nine categories, which are: 1. Cytokine biomarkers, 2. Coagulation biomarkers, 3. Acute phase protein biomarkers, 4. Receptor biomarkers, 5. Cell marker related biomarkers, 6. Vascular and endothelial damage biomarkers, 7. Vasolidation related biomarkers, 8. Organ dysfunction biomarkers, 9. Genetic biomarkers.

## 2. Cytokine biomarkers

Cytokines participate in the pro-inflammatory as in the inflammatory cascade of sepsis and septic shock. The release of pro-inflammatory mediators characterizes the initial phase of sepsis. Persistence of the latter provokes acquired immunodepression, related to an anti-inflammatory profile, and hence to a delayed decrease in hypersensitivity, an incapacity to cope with the infection and the onset of inflammation. The first human defence mechanism which is activated is the intrinsic immune system (innate immune system). It is the first line of defence and it is motivated by both exogenous (e.g. Gram-, Gram + bacteria) and endogenous factors (e.g. Heat shock protein, direct cellular injury, ischemia-reperfusion). Both in SIRS and sepsis, there is an excessive production of pro-inflammatory cytokines, adhesion molecules, vasodilating mediators and reactive oxygen species, therefore they are detectable in blood, where they are normally absent. However, the circulating cytokines are merely the tip of the iceberg and leukocyte-associated cytokines can be identified even when amounts in plasma are undetectable. Pro-inflammatory responses lead to the activation of successive biological reactions which include: Inflammatory mediators (cytokines, chemokines and lipid mediators) and coagulation / fibrinolysis system.

Cytokines act as inflammatory mediators and regulators of both the immune and inflammatory reactions. Pro-inflammatory cytokines are released in a cascade, with TNF-a and IL-1β being the initial cytokines. These cytokines stimulate the production of other cytokines such as IL-6, IL-8, IL-10 and MIP-1a (macrophage inflammatory protein-1a). TNFa, IL-1b, IL-6, IL-8 and IL-10 are linked to morbidity and mortality in septic patients. IL-6 levels in plasma rise according to sepsis severity, but that does not always happen. The median levels of IL-6 in patients with SIRS are approximately 10-fold higher than that of healthy people, in sepsis are approximately 20-fold higher, in severe sepsis 60-fold higher and in septic shock are 100-fold higher. But, in some patients IL-6 levels are very similar to those of healthy people (3pg/ml). (Socha et al, 2006, Halter et al, 2005, Akira et al, 2003, Mokart et al, 2002, Paterson et al, 2000, Hack et al, 1997, Casey et al, 1993, Pinsky et al, 1993).

IL-6 is induced by TNF-a and has a longer half-life, reliably measured in blood after insult to the host. IL-6, as a marker of infection, is relatively nonspecific as it is elevated in a variety of inflammatory states. IL-6 is one of the initial cytokines released in inflammation, as mentioned above, and may be an early predictor of more downstream effects, such as organ dysfunction. IL-6 may perform well as a diagnostic and prognostic tool of sepsis. IL-6 levels of more than 1000 ng/mL are highly predictive of sepsis related death. The diagnostic and prognostic accuracy of IL-6 may depend on time and frequency of measurement and underlying illness severity, perhaps suggesting the importance of trending cytokine levels with clinical course and therapy As IL-6, IL-8 is not so a good indicator of systemic infection, although elevated IL-8 levels are indicative of multiple organ failure and an increased likelihood of death. Measurement of IL-6 and IL-8 is not optimal for discriminating patients with infectious from those with noninfectious conditions (Chiesa et al, 2003, Iglesias et al, 2003, Harbarth, 2001, Muller et al, 2000, Panacek, 1999, Martin, 1997, De werra, 1997).

Tumor Necrosis Factor-a (TNF-a), which is mainly produced by macrophages, lymphocytes and fibroblasts, causes fever and elevated liver enzymes among others and activates three

main cell signaling pathways, which lead to inflammation and cell death, by binding to its receptor. Plasma levels of TNF-a are increased rapidly after infection and that's how TNF-a activates the inflammation cascade. Additionally, TNF-a levels in plasma are increased due to the elevated production of Haemoxygenase-1 (HO-1), which is implicated by lung injury and organ perfusion (Socha et al, 2006, Kalil et al, 2004, Kan et al, 1999). There are two types of TNFa receptors: TNF-R type 1 (CD120a) – which binds only to TNFα- and TNF-R type 2 (CD120b) – which binds both to TNFα and TNFβ. The three cell signaling pathways, leading to inflammation, activated by TNF-a are the following:

1. TRADD- RIP- TRAF2 pathway stimulates NF-κb pathway and production of antiapoptotic factors, such as Bcl-2.
2. TRADD- RIP-TRAF2-ASK1-MAPK pathway leads mainly to AP-1 production and downstream to the production of pro-apoptotic factors and cell proliferation and cell maturation.

TNFR- CD40 TRADD- FADD pathway leads to cell apoptosis by triggering caspase family cascade (Jaffer et al, 2010) (**Figure 2**).

Cytokines also participate in anti-inflammatory cascade. When the inflammatory response is triggered, anti-inflammatory mechanisms are simultaneously activated. Elevated production of pro-inflammatory cytokines causes the simultaneous production of specific cytokines such as IL-4, IL-10, IL-13, soluble TNF receptor and reduction of lymphocyte cells production. Th1 lymphocytes secrete TNF-a, IL-2 and IFN-γ, while T17 lymphocytes secrete IL-17 leading neutrophils to the infection field, unlike Th2 lymphocytes secrete IL-4, IL-6 and IL-10, while regulatory T-cells (Treg) mediate cytokine secretion amplifying the anti-inflammatory response. Especially IL-10 -produced also by monocytes- is suspensory to Th1-cytokines secretion (and mainly to TNF-a) and inhibits the NF-kB JAK-STAT pathway. IL-13 is secreted by Th-cells and leads to elevated production of IgE, matrix-metalloproteinases (MMPs) and neutrophils while it regulates TNF-a and leucocyte response to SIRS. TNF-a and IL-10 levels are found increased in patients with bad prognosis and the increase is higher in patients with positive blood culture. IL-10 levels can be used to discriminate sepsis from sever sepsis (Jaffer et al 2010, Giamarellos- Bourboulis 2010, Freitas et al 2009, Sobieski et al 2008, Van der Poll et al 2008, Munford et al 2001, Rodriguez-Gaspar et al 2001, Socha et al 2006, Whitlock et al 2006).

## 3. Coagulation biomarkers

Macrophage Migration Inhibitory Factor (MIF) has a critical role in activating the immune system and accelerating the pro-inflammatory response in sepsis (that is why it is thought that in the near future MIF will be a significant target in the treatment of sepsis). Tissue factor activates coagulation cascade (**figure 3**) and fibrinolysis system. Antithrombin III plays a dominant role among antithrombotic and fibrinolytic agents. Antithrombin III is involved in the formation of a complex constituted by activated FVIIa, FIXa, Fxa and FXIa factors and kallikrein. When antithrombin III is inhibited e.g by prostacyclin, Disseminated Intravascular Coagulation (DIC) is caused -which is considered as an important factor in Multi Organ Dysfunction (MODS) development because its normal function is to lower platelet adhesion and activate monocytes. Finally, we must refer to protein C or autoprothrombin IIA, which protects cells from sepsis and it has strong anticoagulative effects through inactivating Va and VIIIa factors of the coagulation system (Mosnier et al, 2007, Hotchkiss et al, 2003, Nicolaes et al, 2003, Mather et al, 1996).

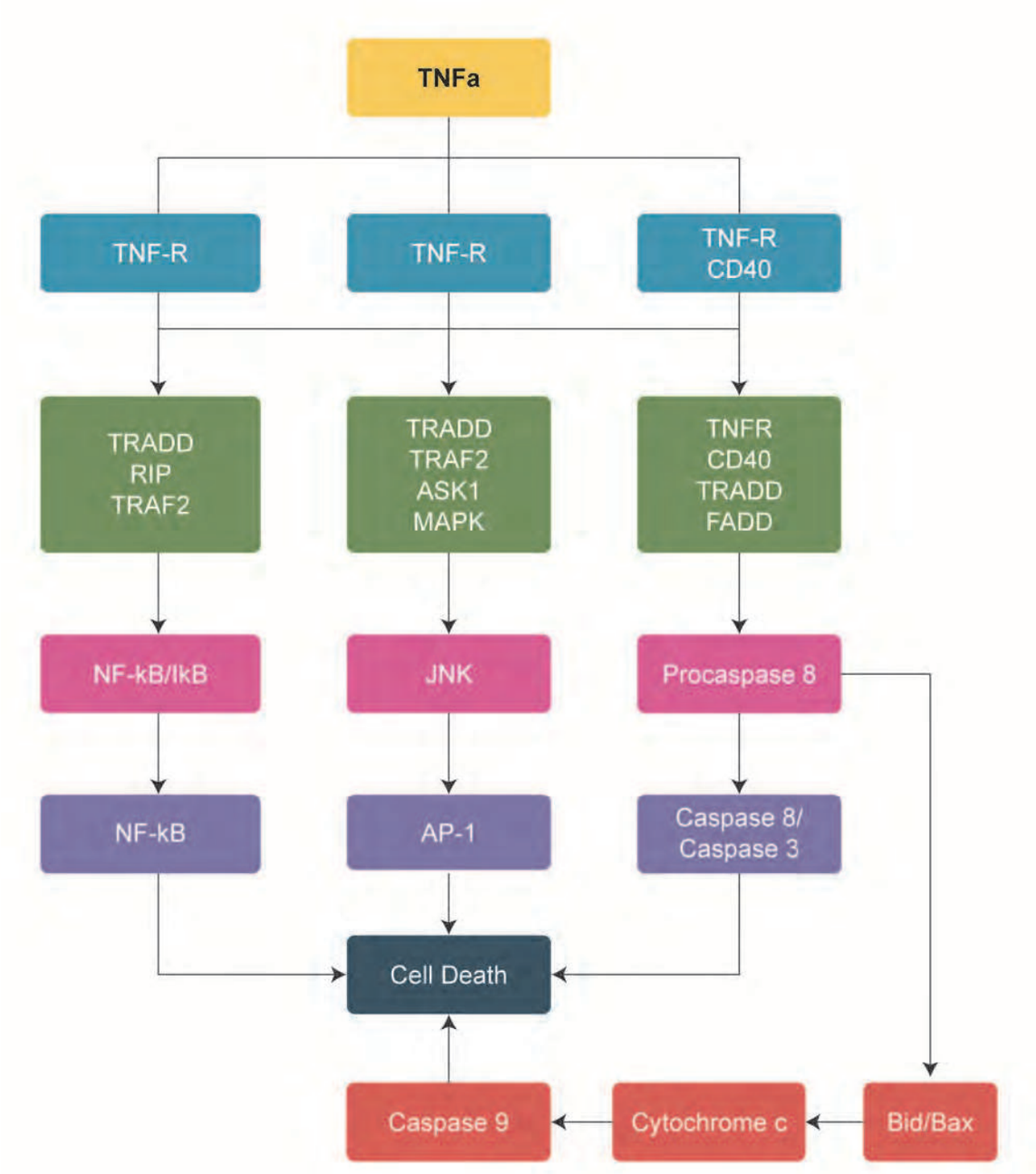


Fig. 2. TNFa activated cellular pathways

The more interesting coagulation factors for sepsis, whose levels have been proposed to be in accordance to sepsis severity and have some prognostic use for septic patient, are easily counted in clinical practice. Activated partial thromboplastin time (aPTT) measurement waveform has been correlated to sepsis. An atypical biphasic transmittance waveform due to decreased light transmission has been observed and it is more common in patients with sepsis. This phenomenon may be due to the formation and precipitation of a calcium dependent complex among C-reactive protein (CRP) and very low density lipoprotein

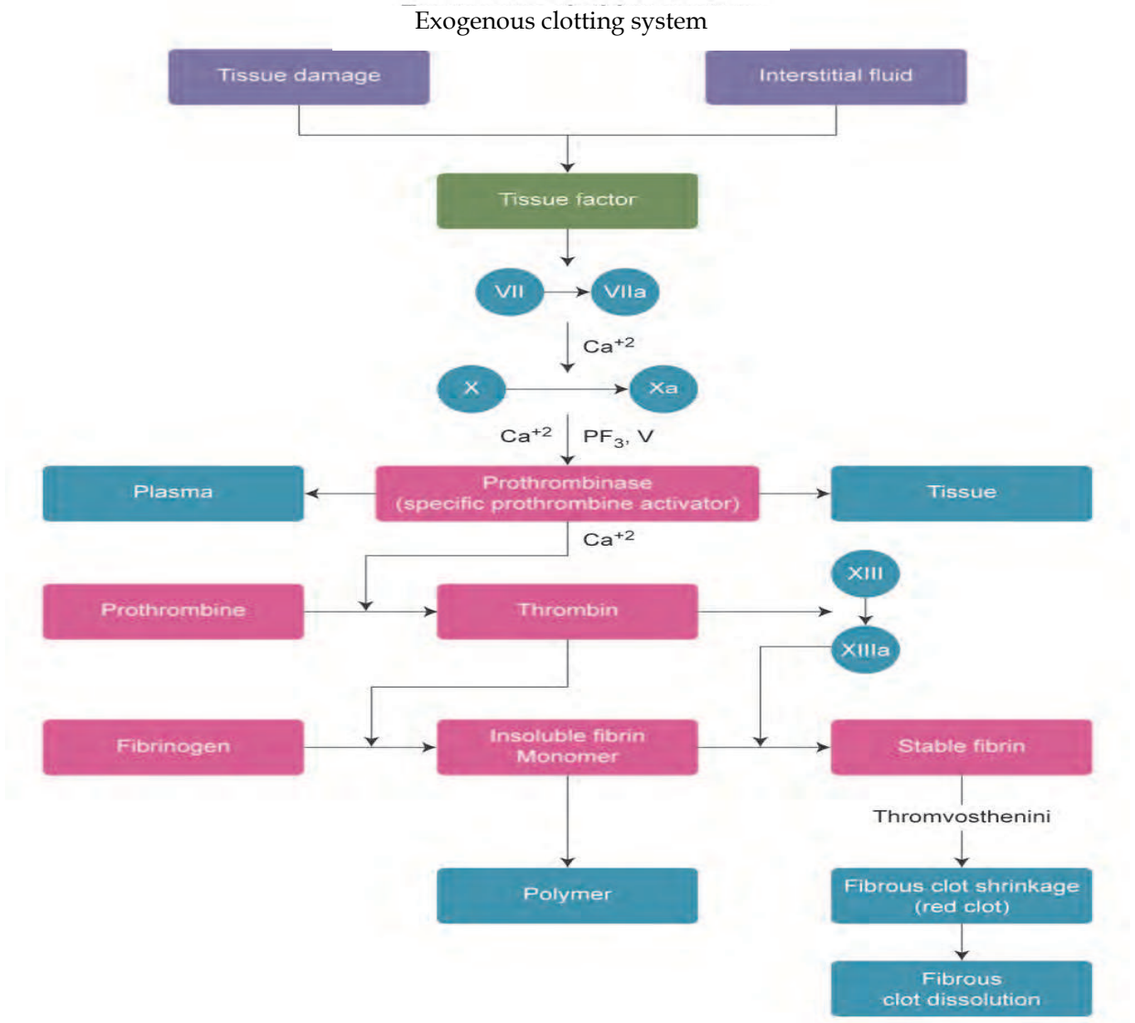


Fig. 3. The exogenous clotting system

(VLDL) in serum of septic patients. That waveform is correlated with negative prediction, sepsis induced disseminated intravascular coagulation and multiorgan failure. Plasminogen activator inhibitor-1 high levels have predictive value for the development of multiorgan failure in septic patients and they are strong correlated with bad prognosis of septic patients with sepsis induced disseminated intravascular coagulation especially when plasminogen activator inhibitor levels are higher than 90 ng/ml. Antithrombin III concentration has been proposed as a prognostic factor, as its levels are lower in septic patients who are not possible to survive. Low protein C levels in septic patient's blood are indicative of increased risk of morbidity and mortality (Zakariah et al, 2008, Madoiwa et al, 2006, Petilla et al, 2002, Fisher et al, 2000).

## 4. Acute phase protein biomarkers

Acute phase protein biomarkers have been more extensively correlated with prognosis of sepsis, compared to other biomarkers. The two main acute phase protein biomarkers which

are related to sepsis are procalsitonin (PCT) and C-reactive protein (CRP). PCT has a bigger sensitivity and specificity in prognosis of sepsis compared to CRP, but CRP is more available and most widely used in clinical practice. Also, PCT is generally elevated in various conditions correlated with inflammatory response, whether CRP has a better prognostic value in the response of therapy in septic patients. However, there are contradictory views for the utility of both biomarkers in prognosis of sepsis and further clinical studies must be demonstrated in this direction. Serum CRP may be used as a biomarker as its concentrations increase in response to inflammation, its half-life is short but its kinetics are not as good as those of PCT. CRP levels are increased in sepsis, but its use as a diagnostic biomarker is not yet established. CRP is proposed to be used as a discriminating factor between septic from non septic shock while it is less accurate than PCT in SIRS from sepsis differentiation. CRP is a poor predictive biomarker of sepsis outcome. Many experimental and clinical trials suggest that PCT is a specific marker for severe bacterial infection and it may be used to distinguish patients who have sepsis from patients who have SIRS in clinical routine. PCT has been shown to be a better biomarker than IL-6 and CRP in characterizing noinfectious from infectious acute respiratory distress syndrome. PCT remains among the most promising biomarkers in sepsis (Jensen, 2006, Castelli, 2004, Clec'h, 2004, Povoa, 2004, Luzzani, 2003, Claeys, 2002, Harbarth, 2001, Muller, 2000, Assicot, 1993).

Other acute phase protein biomarkers which are widely used in clinical practice, are ceruloplasmin - which is correlated with great liver dysfunction in septic patients- and hepcidin which is shown significantly elevated in septic patients and patients with chronic renal failure. Also, another critical biomarker of this category is serum amyloid A (SAA) which is associated with the elevated levels of CRP in septic patients. Finally, there must be a reference on lipopolysacharide binding protein (LBP), which is generally elevated in septic patients compared to the healthy population, but –on the other hand- cannot be used as a prognostic marker in the development of sepsis. (Li et al 2009, Cicarreli et al 2008, Schmit et al 2008, Couto et al 2007, Seller-Perez et al 2005, Oude Nijhuis et al 2003, Becker et al 2008).

## 5. Receptor biomarkers

Many cell receptors have been proposed to have prognostic value for septic patients. Their reliability for clinical routine use is under investigation and only some experimental findings are indicative of receptor biomarkers quality for usage in septic patient diagnosis. Receptor biomarkers that will be further discussed are IL-2 receptor, Toll-like receptor (TLR2 and TLR 4), TNF receptor and TREM-1 receptor. However, many other receptors have been investigated and proposed as possible biomarkers of sepsis, such as CSL2, Fas Receptor and group II phospholipase A2 receptor. Increased serum levels of soluble IL-2 receptor in patients with gram-negative sepsis may behave as predictive indices of shock. Soluble IL-2 receptor is released in biological fluids mostly from T and B lymphocytes that seem to participate in pathogenesis of sepsis. Soluble IL-2 receptor measurement can be easily performed by the use of diagnostic kits and it is suggested to be routinely measured in sera from patients with gram-negative sepsis in order to identify those, who have the strongest risk to develop septic shock. Microorganisms consist of conserved sequences called Pathogen-Associated Molecular Patterns (PAMPs) such as endotoxin/ lipopolysaccharide (LPS) which are located in Gram- negative bacteria cell wall. These PAMPs bind to specific Pattern Recognition Receptors (PRRs) (there are 15 known subtypes), named Toll-like receptors (TLRs) and especially TLR2 and TLR4. TLRs have an

intracellular domain, homologous with IL-1 and the IL-18 receptors. Adaptor proteins facilitate binding to IL-1 receptor-associated kinase, which in turn induces TNF receptor-associated factor-6, leading to nuclear translocation of nuclear factor-kB (NF-kB) and ultimately to activation of cytokine gene promoters, resulting in advanced proinflammatory cytokine production (Rittisch et al, 2008, Brunn et al, 2006, Cornell et al, 2005, Delogu et al, 1995, Willatts et al, 1994). Initially in inflammatory response, LPS is bound by a lipopolysaccharide binding protein which is located to CD14 and TLR4. TLR4 and CD14 are receptors of macrophages and circulating monocytes and they are activated by LPS binding. Their activation causes signal transduction through Toll Inerleukin-1 Receptor (TIR) domain which is referred as myeloid differentiation protein 88. Afterwards, the IL-1 receptor-associated kinase (IRAK) is activated and in turn activates the TNFa-receptor associated factor (TRAF) and the TRAF-associated kinase (TAK). That is the role of TLRs in sepsis. But what is their diagnostic use as biomarkers, if there is any? TLR-2 and TLR-4 expressions are relatively increased in septic critically ill patients, as it was found in experiments using mice. Moreover, the increased levels of those two TLRs were related to advanced possibility to death.

In septic patients, soluble TNFRs plasma levels have been found increased. In the same study it was suggested that the increased levels of the receptor correlate in the same way with multiple organ failure, as with mortality. Elevated soluble TNFR levels may be used as a biomarker for severity of sepsis, in a predicting manner. But more information and clinical trials are needed in order TNFR to be used in clinical routine. Soluble TREM-1 is a reliable marker of bacterial infection, while it participates in the septic process. A decrease in soluble TREM-1 concentration may be indicative of treatment effectiveness. On the contrary, when sTREM-1 levels are persistenly increased the therapy administered to patient is not effective or patients are not in a good condition. Moreover, sTREM-1 concentration is indicative of the prognosis of the patients and they can be used in predicting septic patient outcome. The receptors levels on the 28$^{th}$ day from diagnosis are significantly different in patients with good prognosis from those with not such a good prognosis, which may lead them to death (Rittisch et al, 2008, Annane et al, 2005, Gibot, 2005, Williams et al, 2003, McCuskey et al, 1996 Ertel, 1994).

## 6. Cell marker related biomarkers

Cells that bear on their membrane surface special proteins have been used in experiments in sepsis trials, in order to find some special patterns of expression of those proteins during sepsis and try to combine these findings with sepsis diagnosis and prognosis. So, CD10, CD11, CD14, CD18, CD25, CD28, CD40, CD48, CD54, CD60, CD80 and CD163 are the cell marker proteins which levels have been correlated to sepsis and septic shock prognosis. From those CD10 and CD11c are found in decreased levels in septic patients. Neutrophil CD11b and CD64 appear to be promising markers for diagnosis of early and late onset infections. CD11b is normally expressed in low concentration on the surface of neutrophils and its expression is increased 2-4 times more in infants and adults with positive blood culture for sepsis. CD11b seems to be very useful in early diagnosis of neonatal sepsis. CD14, CD25, CD28, CD40 and CD163 are significant different between septic patients with good prognosis and those with very bad prognosis in the 28$^{th}$ day from sepsis diagnosis. CD69 and CD48 are increased in septic patients (Giamarelos et al, 2010,Nolan et al, 2008, Kaneko et al, 2003, Mishra, 2006).

## 7. Vascular and endothelial damage dysfunction biomarkers

Activated macrophages in sepsis produce early response cytokines TNF-a and IL-1 which stimulate vascular endothelial cells to express Intercellular Adhesion Molecules-1 (ICAM-1), E-selectin and tissue factor. In addition, the adhesion of neutrophils to endothelium involves interaction between endothelial ICAM-1 and beta2 integrin of neutrophils. Adhesion molecules expression is increased in the most severely ill septic patients and it reaches its peak in patients with Multi Organ Failure (MOF). According to these findings, vascular cell adhesion molecule (VCAM-1), E-selectin, P-selectin and endocan are fine prognostic biomarkers in the development of multi-organ dysfunction (MODS) and septic shock in septic patients. Also, L-laminin shows a significant increase in septic patients and ADAMTS-13 is increased in septic patients without disseminated intravascular coagulopathy (DIC) compared to septic patients with DIC. Additionally, there is an existing increase in other biomarkers of this category like cellular and soluble endothelial leukocyte adhesion molecule (ELAM)-1 and vascular endothelial growth factor (VEGF) in septic patients with very poor prognosis, but further clinical studies must be demonstrated. (Drake et al 1993, Mimuro et al 2008, Seidelin et al 2002, Lopez et al 1999, Whalen et al 2000, Cowley et al, 1994, Moss et al, 1996).

## 8. Vasolidation related biomarkers

There are several biomarkers of sepsis related to vasodilation which have been already identified in patients. Firstly, C-type natriuretic peptide (CNP), anandamide, angiotesin converting enzyme (ACE), 2- arachidonoglykerol and neuropeptide Y are shown to be significantly increased in septic patients comparing to the healthy population, although further clinical studies must be done to ensure this finding. Also, 47 kD high molecular weight kininogen (HK) shows a critical correlation with more severe sepsis. Furthermore, proadrenomedulin and andrenomedulin are correlated with development of sepsis. But, the most critical vasodilatory factor which is correlated with development of sepsis is nitric oxide and its derivatives. In the metabolic pathway of inducible (or induced) NO synthase (Inducible Nitric Oxide Synthetase: iNOS), the inducible NO synthetase overproduces nitric oxide by reacting with L-Arginine, which results to relaxation of smooth muscle cells. Its concentration increases as a result of gene activation, which in turn produces high levels of nitric oxide (NO). NO activates guanylyl cyclase enzyme. Guanylyl cyclase increases the production of cyclic guanosine monophosphate (cGMP). However, if NO production is inhibited by blockading the NOS enzyme, then it is associated with poor prognosis in septic patients. Also, nitric oxide is being hugely produced after the activation of iNOS gene by Hypoxia Inducible Factor-1 (HIF-1). Finally, there are tetrahydrobiopterin which is shown increased in critically ill septic patients and vasoactive intestinal peptide (VIP) which is shown quite increased in tissues of patients with severe peritonitis. Elastin is shown decreased in septic patients compared to healthy population (Jacob et al 2007, Levy, et al 2005, Faury et al, 2005, Jang et al, 2004, Hama et al 1994, Beer et al 2002, Amalich et al 1995, Deitz et al, 1987).

## 9. Organ dysfunction biomarkers

Specific organ dysfunction biomarkers which are shown significantly increased in septic patients compared to healthy population are myocardial angiotensin II, pancreatitis-associated protein-I, pre B cell colony-enhancing factor (PBEF), glial fibrillary acidic protein

(GFAP) and Gc-globulin. Even most, Gc-globulin - which acts as an actin scavenger- has a good prognostic value in the development of multi-organ dysfunction, as it is found in low concentrations in patients developing sepsis and respiratory failure (Hsu et al 2008, Ji et al 1996, Tribl et al 2004, Ye et al 2005).

In sepsis it is highly possible that the balance between inflammatory and anti-inflammatory mechanisms becomes unbalanced so organ dysfunction and cardiovascular dysfunction, metabolic disturbances, renal dysfunction and haematological dysfunction are possible to happen. All the above clinical conditions combined are leading to multiple organ dysfunction. So, after an insult, the balance between inflammatory response and immunoparalysis determine the prognosis of the septic patient, which makes the above biomarkers of vital importance for them (Paterson et al, 2000, Poderoso et al, 1996, Neumann et al, 1997, Baudo et al, 1998, Karima et al, 1999, Grover et al, 1999,) **(figure 4)**.

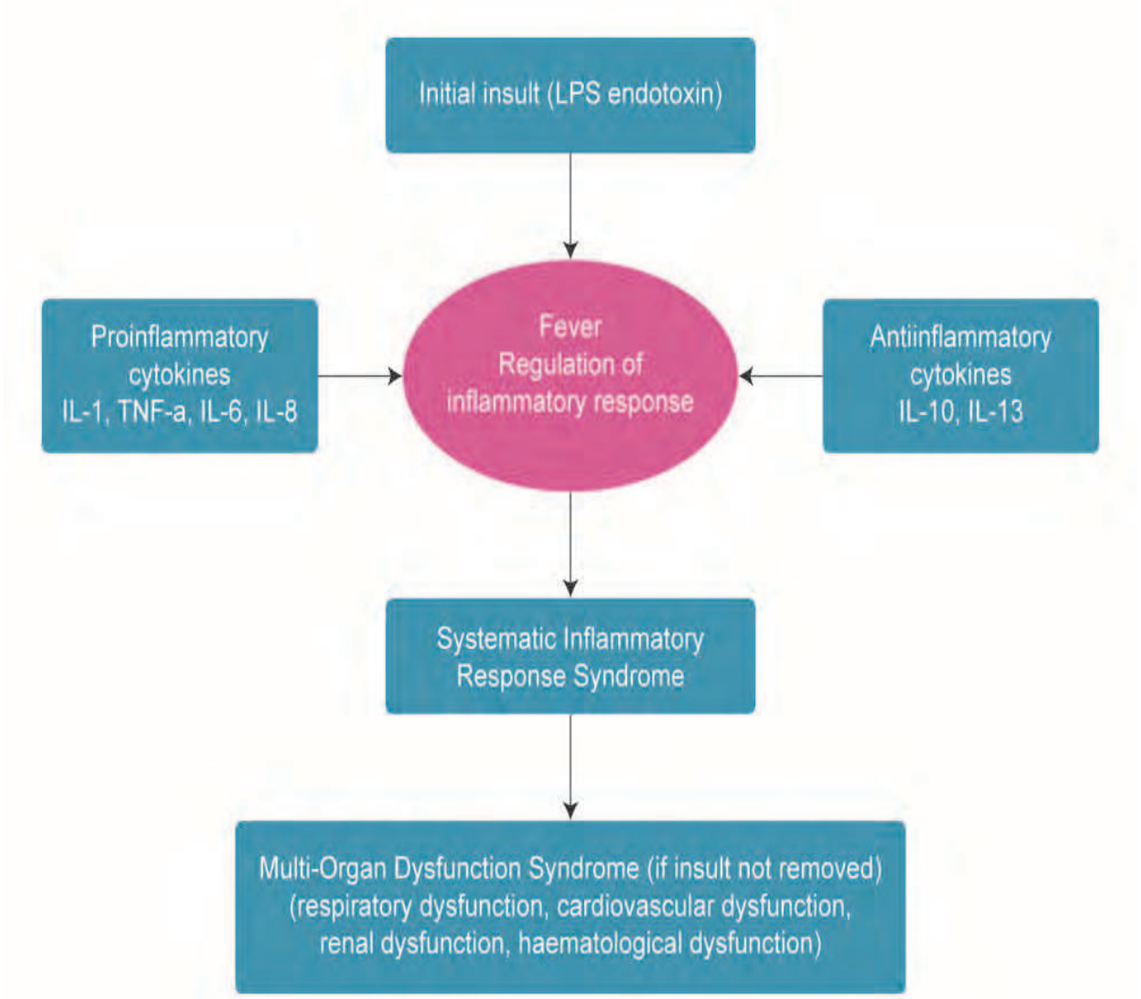


Fig. 4. The process of sepsis

## 10. Genetic biomarkers-single nucleotide polymorphisms

As in almost every other disease, genetic predisposition seems to play an important role in susceptibility in sepsis. Despite modern treatment of sepsis (eg, hydrocortisone) important genetic factors may be responsible even for death by sepsis. Also, along with genetic predisposition, comorbidity and age seem to play a significant role (Alberti et al 2002, Yende et al 2008, Pachot et al 2006, Wong et al 2007, Calvano et al 2005, Annane et al.1998, Bollaert et al.1998, Briegel et al.1999).

In recent years, there have been many clinical studies using modern methods and techniques of molecular genetics, such as genotyping to correlate gene expression with inflammatory response. Specific polymorphisms in some genes express proteins involved in the cascade of systematic inflammatory response and they have an important role in sepsis. These polymorphisms participating in sepsis are Single nucleotide polymorphisms (SNPs). SNPs of a gene is a DNA sequence variation which occurs whenever there is a difference in a single nucleotide position in genome and this one differs between members of a species. The simplest change of this kind is the substitution of one nucleotide by another which is not similar to the previous one. Single nucleotide polymorphisms have a minor allele frequency of >1% (or 0.5% etc.) A large number of SNPs in several genes involved in inflammatory response have been found to vary in their clinical outcomes during infection (Arcaroli et al 2005, Waterer et al 2003, Holmes et al 2003, Stuber et al 1996, Barber et al 2006). The major genetic agents that have been studied are the following:

**Toll-like receptors:** Polymorphisms found in TLR5 and TLR2 are correlated with bacterial infections, and those in TLR4 are correlated with susceptibility in sepsis (Sutherland et al. 2005, Skinner et al 2005, Hawn et al. 2003).

**Collectins:** There are collectins, such as Mannose binding lectine (MBL) whose polymorphisms associate with increased incidence of systemic inflammatory syndrome caused by infectious and noninfectious causes, increased severity of sepsis, and increased mortality although the results of some studies on this topic are contradictory (Garred et al. 2003, Roy et al. 2002, Gong et al 2007, Kronborg et al. 2002).

**Tumor Necrosis Factor-a (TNF-a):** TNF-a gene is polymorphic and there are various polymorphisms in its products. TNF-308 polymorphism with A-allele substitution is of high importance (the most common is G-allele) as it is correlated with increasing risk of death in septic patients. On the other hand, some studies state that there is no correlation between TNF polymorphism and sepsis (O'Keefe et al. 2002, Nakada et al. 2005, Azim et al. 2007, Schueller et al. 2006, Waterer et al. 2001).

**Interleukin-1 (IL-1) Family:** Polymorphisms in IL1β may correlate with sepsis according to its cause, as in some cases they are associated with sepsis development and in some cases they are not. Also, there are conflicting studies of variants of IL-1 receptor antagonist gene (IL-1RN) and its correlation with mortality in patients with septic shock (Arnalich et al. 2002, Ma et al. 2002, Fang et al. 1999, Turner et al. 1997).

**Interleukin-10 (IL-10):** IL-10-1082 G polymorphic allele presence is correlated to sensitivity to pneumonia and susceptibility to septic shock as well as with multiorgan dysfunction (Stanilolva et al. 2006, Gallagher et al. 2003, Schaaf et al. 2003).

Furthermore, SNPs have also been found in genes expressing proteins participating into the inflammatory response. These genes, which SNPs may show correlation with septic shock are: *Lymphotoxin Alpha (LTA) gene*, *Immunoglobin Receptors genes*, *Heat shock protein gene*, *Protein C gene* and *Plasminogen activation inhibitor (PAI) -1 gene*, *Lippopollysaccharide binding*

*protein (LBP) gene*, *CD14 gene* and *Myeloid differentiation protein-2 (MD-2) gene* (Gu et al. 2007, Temple et al. 2004, Yuan et al. 2003, Ye et al. 1995, Binder et al. 2007, Geishofer et al. 2005, Haralambous et al. 2003, Hermans et al. 1999, Van der Poll et al. 2001, Hubacek et al. 2001, Waterer et al. 2001).

As discussed in this chapter there are many biomarkers of sepsis. All of them are very promising in sepsis diagnosis and prognosis, but more evidence is needed so as to be valuable for in every day clinical routine. Sepsis severity and its high incidence to patient's death make extremely difficult every decision in using one and only one clue as the absolute biomarker for sepsis diagnosis and prognosis. Sepsis is such a complex syndrome that every oversimplified approach may become lethal for patients. Multi-biomarker control is the best approach till now. As research continues newer and more advanced biomarkers may be found helping in sepsis diagnosis and therapeutics.

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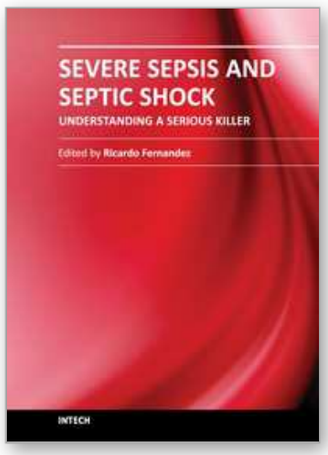


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Despite recent advances in the management of severe sepsis and septic shock, this condition continues to be the leading cause of death worldwide. Some experts usually consider sepsis as one of the most challenging syndromes because of its multiple presentations and the variety of its complications. Various investigators from all over the world got their chance in this book to provide important information regarding this deadly disease . We hope that the efforts of these investigators will result in a useful way to continue with intense work and interest for the care of our patients.

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