

To what extent are the terminal stages of sepsis, septic shock, SIRS, and multiple organ dysfunction syndrome actually driven by a prion/amyloid form of fibrin? bioRxiv preprint.

^{1,2,3}Douglas B. Kell & ⁴Etheresia Pretorius

¹School of Chemistry, ²The Manchester Institute of Biotechnology, and ³Centre for Synthetic Biology of Fine and Speciality Chemicals, The University of Manchester, 131, Princess St, MANCHESTER M1 7DN, Lancs, UK

⁴Department of Physiology, Faculty of Health Sciences, University of Pretoria, Arcadia 0007, South Africa.

Keywords: sepsis – SIRS – LPS – fibrin – dormant bacteria – septic shock – MODS

To what extent are the terminal stages of sepsis, septic shock, SIRS, and multiple organ dysfunction syndrome actually driven by a prion/amyloid form of fibrin?	1
Abstract.....	3
Introduction	4
Normal blood coagulation and coagulopathies.....	6
Endotoxin-induced ‘disseminated intravascular coagulation’	9
Prions, protein free energies, and amyloid proteins	9
Amyloid-like conformational transitions in fibrin(ogen)	9
Inflammatory nature of fibrin	11
Further evidence for the ‘trigger’ role of LPS in large-scale amyloid formation	11
Cytotoxicity of β -amyloids	12
Sequelae consistent with the role of β -amyloids in the ‘sepsis cascade’ to organ failure and death	12
How might this understanding lead to improved treatment options?	12
Concluding remarks	13
Acknowledgments.....	13
Ethical approval disclosure	14
Conflict of interest	14
Legends to figures.....	15
References	16

Abstract

A well-established development of increasing disease severity leads from sepsis through septic shock, SIRS, multiple organ dysfunction syndrome and cellular and organismal death. We argue that a chief culprit is the LPS-induced anomalous coagulation of fibrinogen to produce a form of fibrin that is at once inflammatory, resistant to fibrinolysis, and underpins the disseminated intravascular coagulation commonly observed in sepsis. In particular, we argue that the form of fibrin produced is anomalous because much of its normal α -helical content is transformed to β -sheets, as occurs in established amyloidogenic and prion diseases. We hypothesise that these processes play a major role in the passage along the above pathways to organismal death, and that inhibiting them would be of great therapeutic value, a claim for which there is emerging evidence.

Introduction

Sepsis is a disease with high mortality [1-4]. However, the original notion of sepsis as the invasion of blood and tissues by pathogenic microorganisms has long come to be replaced, in the antibiotic era, by the recognition that in many cases the main causes of death are not so much from the replication of the pathogen *per se* but from the host's 'innate immune' response to the pathogen (e.g. [5-8]). In particular, microbial replication is not even necessary (and anyway most bacteria in nature are dormant [9-13]), as this response is driven by very potent [14] inflammagens such as the lipopolysaccharides (LPS) of Gram-negative bacteria and equivalent cell wall materials such as lipoteichoic acids from Gram-positives [15]. To this end, such release may even be worsened by antibiotic therapy [16-19]. In unfavourable cases, this leads to an established cascade (Fig 1) [20] in which the innate immune response, involving pro-inflammatory cytokines such as IL-6, IL-8, MCP-1, TNF- α and IL-1 β [21], becomes a 'cytokine storm' [22-26] leading to a 'systemic inflammatory response syndrome' (SIRS) [27-32], septic shock [3], apoptotic [33] and necrotic [34] cell death, multiple organ failure [35] (MOF, also known as Multiple Organ Dysfunction Syndrome, MODS [36; 37]), and finally organismal death. All of the above is well known, and may be taken as a non-controversial background.

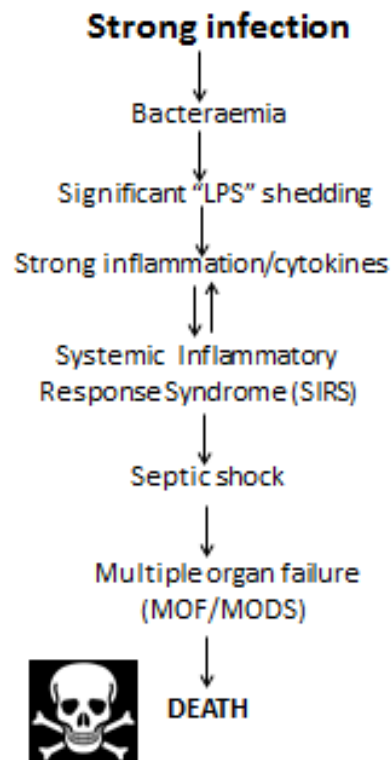


Figure 1: A standard cascade, illustrating the progression of infection through sepsis, SIRS and death.

Most recently [38], definitions of sepsis have come to be based on organ function and the Sequential (Sepsis-Related) Organ Failure Assessment (SOFA) Scores [39]. These latter take into account the multisystem nature of sepsis, and include respiratory, coagulatory (but only based on platelet counts), hepatic, cardiovascular, renal and CNS measurements. A SOFA score of 2 or greater typically means at least 10% mortality. Specifically, sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is defined as a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to increase mortality substantially.

Table 1 (based on [39]) shows the potential values that contribute to the SOFA score. *catecholamine and adrenergic agents administered for at least 1h; doses in $\mu\text{g} \cdot (\text{kg} \cdot \text{min})^{-1}$.

SOFA score	1	2	3	4
<i>Respiration</i> PaO ₂ /FiO ₂ (mm Hg)	<400	<300	<200 (with respiratory support)	<100 (with respiratory support)
<i>Coagulation</i>	<150	<100	<50	<50

10^{-3} .platelets.mm ⁻³				
<i>Liver</i> Bilirubin mg.dL ⁻¹ (μ M)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (>204)
<i>Cardiovascular</i> Hypotension	MAP<70 mm Hg	Dopamine $\leq 5^*$ or Dobutamine (any dose)	Dopamine >5 or epinephrine ≤ 0.1 or norepinephrine ≤ 0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
<i>CNS</i> Glasgow Coma Score	13-14	10-12	6-9	<6
<i>Renal</i> Creatinine, mg.dL ⁻¹ (μ M) or urine output	1.2-1.9 (110- 170)	2.0-3.4 (171-299)	3.5-4.9 (300-440) Or <500ml.d ⁻¹	>5.0 (>440) or <200ml.d ⁻¹

Absent from Fig 1 and the usual commentaries of this type are any significant role of coagulopathies, although these too are a well-established accompaniment of SIRS/sepsis [40-49], and they are our focus here. They form part of an emerging systems biology story (e.g. [13; 50-57]) in which iron dysregulation and an initially minor infection is seen to underpin the aetiology of many chronic inflammatory diseases normally considered (as once were gastric ulcers [58]) to lack a microbial component.

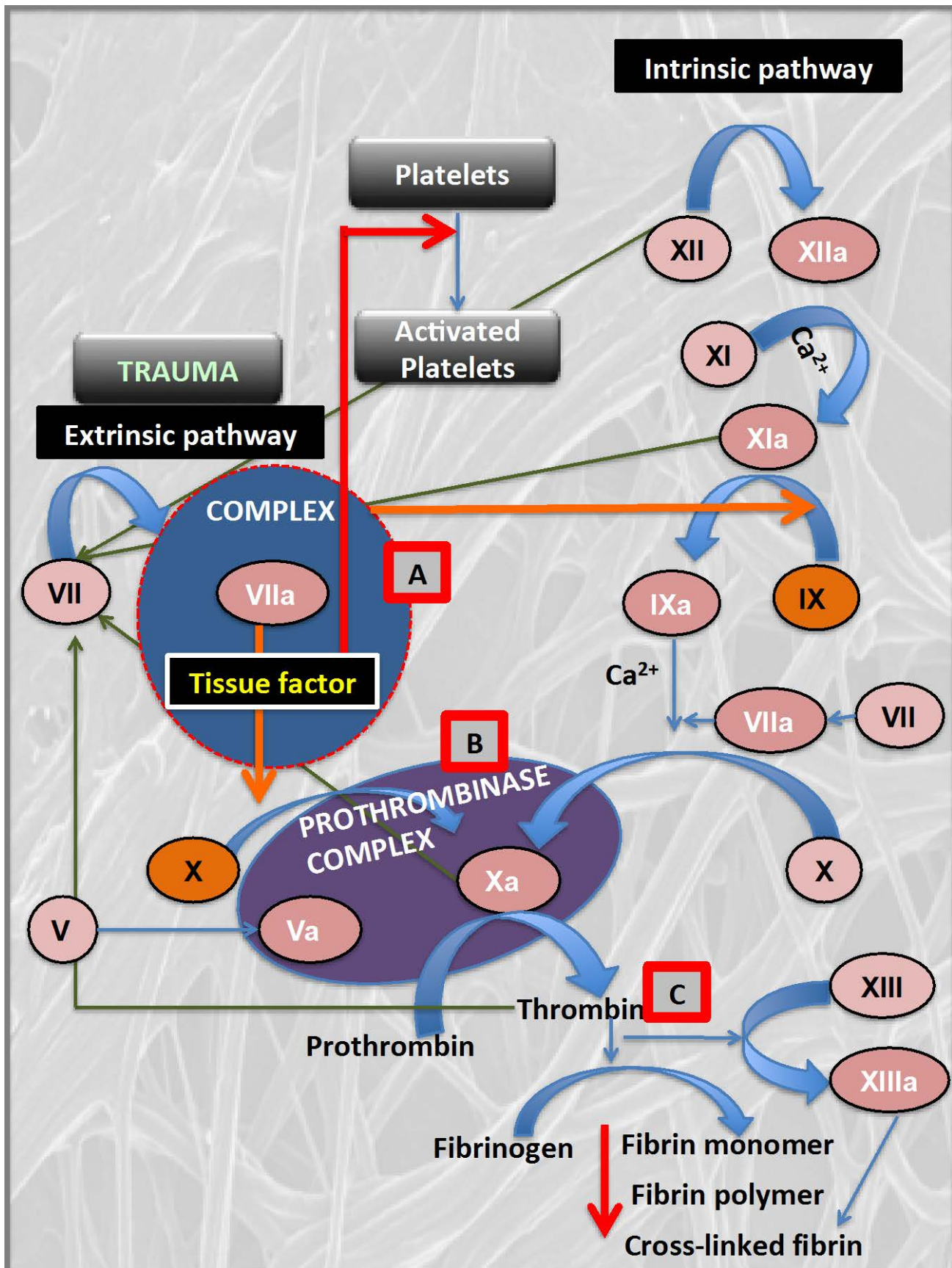
Here we develop the somewhat different case for those conditions that are recognised as involving a genuine initial microbial invasion and sepsis and inflammation driven (in particular) by the cell wall components of bacteria, although we note that the same kinds of arguments apply to viruses [59] and to other infections.

Normal blood coagulation and coagulopathies

There are two main pathways of activation of 'normal' blood coagulation to form a clot, as occurs e.g. in response to wound healing. They have been expertly reviewed many times (e.g. [60-65]), are known as 'intrinsic' and 'extrinsic', and are diagrammed in Fig 2. In short, after damage to e.g. blood vessel, collagen is exposed and **factor VII** leaves the circulation that comes into contact with tissue factor (TF), forming a complex called TF-FVIIa (complex shown in blue (block A) in Fig 2). This complex activates both factors IX and X (shown in orange). **Factor VII** is also activated by various molecules, including **thrombin**, **factor XIa**, **factor XII** and **factor Xa** (green arrows in Fig 2). Factor Xa and its co-factor Va form the prothrombinase complex (shown in purple (block B) in Fig 2) and activates thrombin via

prothrombin (block C in Fig 2). Finally, the terminal stages of the coagulation pathway happens, where a cross-linked fibrin polymer is formed as a result of fibrinogen (typically present in plasma at $2-4 \text{ g.L}^{-1}$) conversion to fibrin and cross-linking due to the activation of factor XIII, a transglutaminase. Thrombin activates factor XIII into factor XIIIa which finally acts on fibrin to form the cross-linking between fibrin molecules to form an insoluble fibrin clot. This fibrin clot, when viewed under a scanning electron microscope, consists of individually visible fibrin fibres, discussed in the next paragraphs (see Fig 3A for a representative healthy clot structure, created when thrombin is added to plasma (e.g. [54; 66-68]).

Figure 2: The coagulation pathway, with specific reference to factor VII and the tissue factor and factor VIIA complex, TF-FVIIa (block A) the prothrombinase complex (block B) and the activation and action of thrombin (block C).



The normal picture of fibrinogen polymerisation involves the removal of two fibrinopeptides from fibrinogen, which is normally rich in α -helices, leading to its self-association via knobs and stalks, but with otherwise no major changes in secondary structure.

Coagulopathies occur when the rate of clot formation or dissolution is unusually fast or slow, and in the case of chronic inflammatory diseases these seem largely to coexist as hypercoagulation and hypofibrinolysis, arguably implying a common cause [54]. In a series of papers, we have shown in a number of diseases such as stroke [69-71], type 2 diabetes [68; 72], Alzheimer's [73-75], and hereditary haemochromatosis [67], that instead of their usual 'spaghetti-like' appearance the fibrin clots induced by added thrombin adopted the form of 'dense matted deposits'. The same kinds of effect could also be induced by unliganded iron [67; 76-78], although no molecular explanation was (or could be) given.

Endotoxin-induced 'disseminated intravascular coagulation'

Endotoxin (LPS) may also induce a runaway form of hypercoagulation (e.g. [41; 79-93]) known as disseminated intravascular coagulation (DIC). There is significant evidence, now that DIC is reasonably well defined [37; 94-96], that it can lead directly to multiple organ failure and death ([97], and see below). We hypothesise here that the form of clotting in DIC in fact involves autocatalytic β -amyloid formation (which is consistent with the faster clot formation in the presence of endotoxin [73]), and that this in particular is a major contributor to the various stages of sepsis, SIRS, MODS and ultimately organismal death.

Prions, protein free energies, and amyloid proteins

Although it was originally shown that at least some proteins, when denatured and renatured, could revert to their original conformation [98; 99], implying that this was (isoenergetic with) the one of lowest free energy, this is now known not to be universal. Leaving aside chaperones and the like, one field in which proteins of the same sequence are well known to adopt radically different conformations, with a much more extensive β -sheet component (that is indeed thermodynamically more stable) is that of prion biology [100; 101]. The PrP^c and PrP^{sc} conformations, and the catalysis of the conversion to itself by the latter of the former are very well known. The key point for us here, however, is indeed that this definitely implies (e.g. [100-107]) that proteins that may initially fold into a certain, ostensibly 'native', conformation can in fact adopt stable and more β -rich conformations of a lower free energy, separated from that of the original conformation by a potentially significant energy barrier.

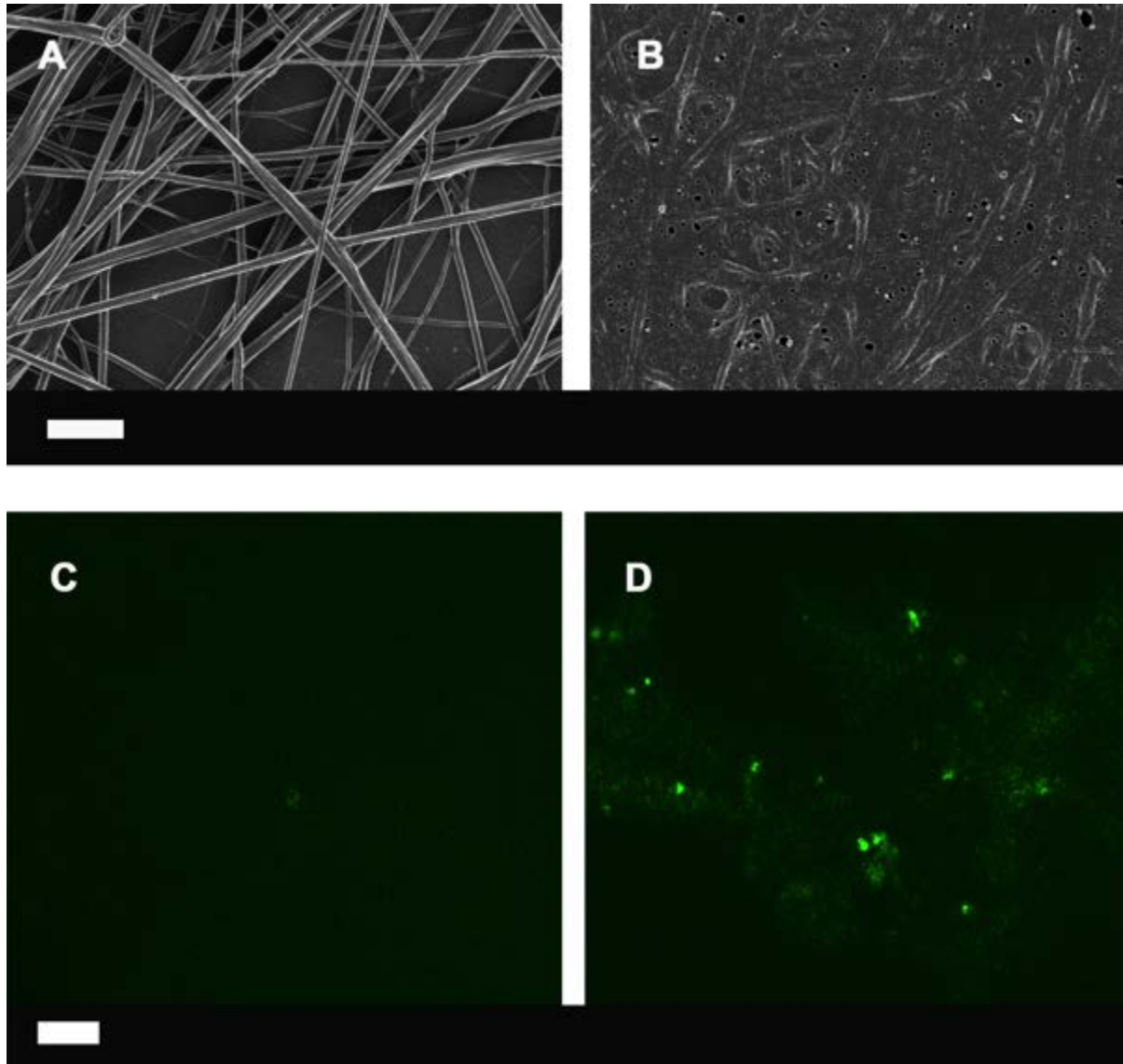
Amyloid-like conformational transitions in fibrin(ogen)

As mentioned, the general view is that no major secondary structural changes occur during normal fibrin formation. However, we know of at least three circumstances in which fibrin can

(i.e. is known to) adopt a β -rich conformation. These are (i) in the case of specific mutant sequences of the fibrinogen α chain [108-114], (ii) when fibrin is stretched mechanically beyond a certain limit [115-121], (iii) when formed in the presence of certain small molecules, including bacterial lipopolysaccharide (LPS) [57; 122; 123]. Thus it is well established that fibrin can form β -rich amyloids, although it is assumed that conventional blood clotting involves only a ‘knobs and stalks’ mechanism without any major changes in secondary structure (e.g. [60-65; 124; 125]. We hypothesise here that the ‘dense matted deposits’ seen earlier are in fact β -rich amyloids, and that it is this coagulopathy in particular that contributes significantly to the procession of sepsis along or through the cascade of toxicity outlined in Fig 1.

In particular, thioflavin T (ThT) is a stain whose fluorescence (when excited at 440-450nm or so) is massively enhanced upon binding to β -rich amyloids (e.g. [126-135]). Fig 3 A,B show SEM pictures of ‘normal’ clots and dense matted deposits, respectively, while Fig 3C,D show how ThT stains clotted platelet-poor plasma from a patient with an inflammatory disease (type 2 diabetes) much more strongly than the equivalent plasma from a healthy control.

Figure 3: The results of thrombin-mediated blood clotting: **(A)** Normal fibrin fibres, **(B)** the same but taken from a patient with type II diabetes **(C)** ThT confocal with no LPS, **(D)** ThT confocal with LPS. Scale bars of **A** and **B**: 1 μm and **C** and **D** 10 μm .



Inflammatory nature of fibrin

The fact that fibrin itself is or can be inflammatory is well established (e.g. [84; 136-141]), and does not need further elaboration; our main point here is that in none of these studies to date has it been established whether (or to what extent) the fibrin is in a β -amyloid form or not.

Further evidence for the 'trigger' role of LPS in large-scale amyloid formation

In our previous studies [122], we found that LPS (endotoxin) at a concentration of just $0.2\text{ng}\cdot\text{L}^{-1}$ could trigger the conversion of some 10^8 times more fibrinogen molecules [122], and that the fibrin fibres so formed were β -amyloid in nature. (A very large amplification of structural molecular transitions could also be induced by LPS in a nematic liquid crystal [142-144].) Only some kind of autocatalytic processes can easily explain this kind of polymerisation, just as occurs in prions.

Cytotoxicity of β -amyloids

This is so well known (e.g. [73; 145-153]) as barely to need rehearsing, although the relative toxicities of soluble material, protofibrils, fibrils and so on is less well understood [154], in part because they can equilibrate with each other even if added as a 'pure' component (of a given narrow MW range).

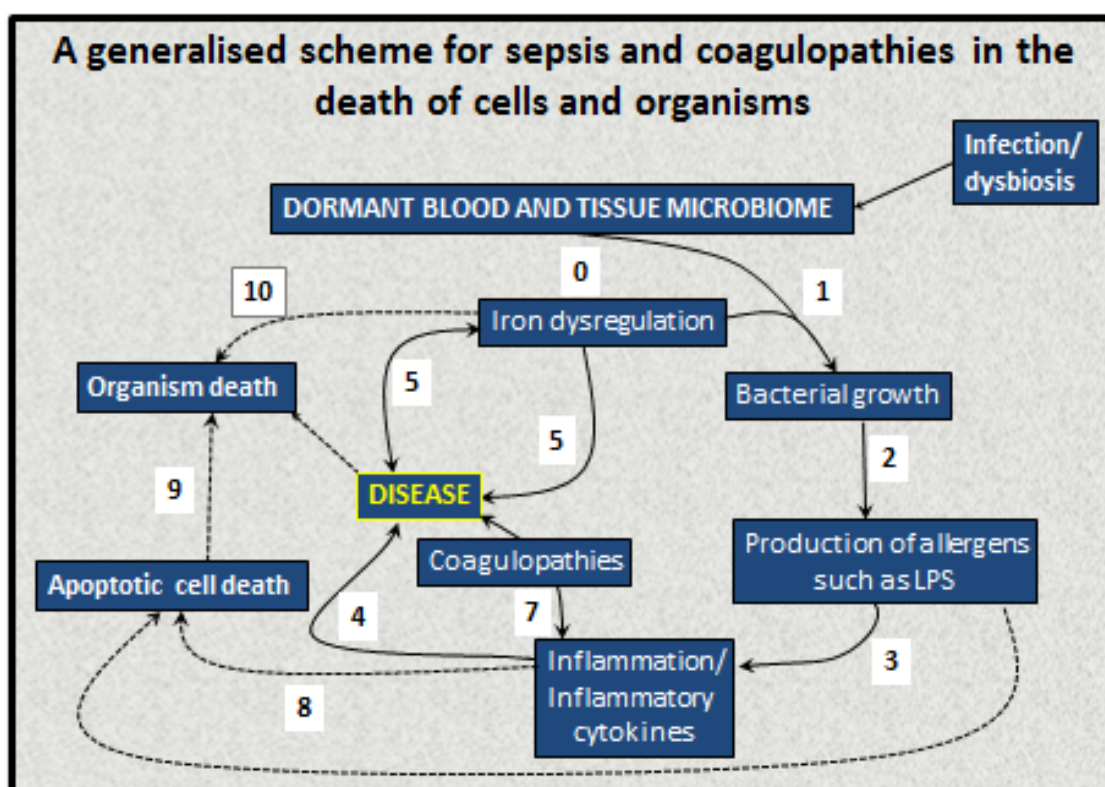
Sequelae consistent with the role of β -amyloids in the 'sepsis cascade' to organ failure and death

If vascular amyloidogenesis really is a significant contributor to the worsening patient conditions as septic shock moves towards MOF/MODS and death, with the cytotoxic β -amyloids in effect being largely responsible for the multiple organ failure, then one might expect it to be visible as amyloid deposits in organs such as the kidney (whether as biopsies or post mortem). It is certainly possible to find evidence for this [155-160], and our proposal is that such amyloid should be sought using thioflavin T staining in autopsy tissue.

How might this understanding lead to improved treatment options?

Over the years there have been many high-profile failures of therapies for various aspects of severe inflammation, sepsis, septic shock, and SIRS. These include therapies aimed at endotoxin itself (Centoxin) [161-163], and the use of recombinant activated protein C [164], and of Drotrecogin alfa [163; 165-167]. Anti-cytokine and anti-inflammatory treatments have also had (at best) mixed results [168; 169].

However, the overall picture that we have come to is given in Fig 4. This implies that we might hope to stop the progress of the sepsis/SIRS/MODS cascade at any (preferably



several [170]) of a number of other places, including via iron chelation [50; 51; 171; 172], the use of anti-inflammatories, of anticoagulants such as heparin [140], and of stimulants of fibrinolysis [173]. The success of heparin [174; 175] (see also [176-178]) is especially noteworthy in the context of the present hypothesis, though it may have multiple (not simply directly anti-coagulant) actions [179; 180]. It is also noteworthy that HDL cholesterol is a protective against sepsis [55; 181-183] (HDL are anti-oxidant [184] and anti-inflammatory [185] and can also mop up endotoxin [186-188]), so the beneficial role of certain statins in sepsis [189; 190] should be seen in the context of their much more potent anti-inflammatory role [50] than any role involved in lowering overall serum cholesterol. Phospholipid emulsions may also serve [191].

Figure 4: A systems biology model of the development of coagulopathies during SEPSIS, SIRS and MODS.

Recombinant soluble human thrombomodulin (TM- α) is a novel anticoagulant drug, and has been found to have significant efficacy in the treatment of sepsis-based DIC [192-201], again adding further weight to our hypothesis. As Okamoto and colleagues [178] point out, “In the European Union and the USA, the 2012 guidelines of the Surviving Sepsis Campaign do not recommend treatment for septic DIC [3; 202]. In contrast, in Japan, aggressive treatment of septic DIC is encouraged [203-205]”, and that “that Japan is one of the countries that most effectively treats patients with septic DIC” [178].

Thus, if it is accepted that the type of fibrin that is formed is substantially of the β -amyloid variety, then anticoagulant and other drugs that inhibit or reverse such amyloid processes should also be of value [206], as they seem to be in Alzheimer-type dementia [207-209].

Concluding remarks

There is by now abundant evidence that coagulopathies involving fibrin clots are a major part of sepsis, SIRS, septic shock, MODS, DIC and organismal death. We have invoked further evidence that the type of fibrin involved is a β -amyloid form, and that it is this that is especially damaging; this definitely needs to be tested further, for instance using appropriate stains [127] and/or X-ray measurements [210] in concert with cellular toxicity assays. Finally, we suggest that anticoagulant therapies that inhibit or reverse those β -amyloid forms of fibrin production will be especially valuable. To this end, lowering the levels of fibrinogen itself would seem to be a desirable aim [211].

Acknowledgments

We thank the Biotechnology and Biological Sciences Research Council (grant BB/L025752/1) as well as the National Research Foundation (NRF) and Medical Research

Council (MRC) of South Africa for supporting this collaboration. This is also a contribution from the Manchester Centre for Synthetic Biology of Fine and Speciality Chemicals (SYNBIOCHEM) (BBSRC grant BB/M017702/1). We thanks Dr Janette Bester for confocal microscopy. DBK thanks Prof Nigel Harper for a useful discussion.

Ethical approval disclosure

Ethical approval was granted at the University of Pretoria for all human studies (Human Ethics Committee: Faculty of Health Sciences): E Pretorius.

Conflict of interest

The authors have nothing to disclose.

Legends to figures

Figure 1: A standard cascade, illustrating the progression of infection through sepsis, SIRS and death.

Figure 2: The coagulation pathway, with specific reference to factor VII and the tissue factor and factor VIIA complex, TF-FXIIa (block A) the prothrombinase complex (block B) and the activation and action of thrombin (block C).

Figure 3: The results of thrombin-mediated blood clotting: **(A)** Normal fibrin fibres, **(B)** the same but taken from a patient with type II diabetes **(C)** ThT confocal with no LPS, **(D)** ThT confocal with LPS. Scale bars of **A** and **B**: 1 μm and **C** and **D** 10 μm .

Figure 4: A systems biology model of the development of coagulopathies during SEPSIS, SIRS and MODS.

References

- [1] Martin, G. S., Mannino, D. M., Eaton, S. & Moss, M. (2003). The epidemiology of sepsis in the United States from 1979 through 2000. *New England Journal of Medicine* **348**, 1546-1554.
- [2] Angus, D. C. & van der Poll, T. (2013). Severe sepsis and septic shock. *N Engl J Med* **369**, 840-51.
- [3] Dellinger, R. P., Levy, M. M., Rhodes, A., Annane, D., Gerlach, H., Opal, S. M., Sevransky, J. E., Sprung, C. L., Douglas, I. S., Jaeschke, R., Osborn, T. M., Nunnally, M. E., Townsend, S. R., Reinhart, K., Kleinpell, R. M., Angus, D. C., Deutschman, C. S., Machado, F. R., Rubenfeld, G. D., Webb, S. A., Beale, R. J., Vincent, J. L., Moreno, R. & Surviving Sepsis Campaign Guidelines Committee including the Pediatric Subgroup. (2013). Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* **41**, 580-637.
- [4] Fleischmann, C., Scherag, A., Adhikari, N. K., Hartog, C. S., Tsaganos, T., Schlattmann, P., Angus, D. C., Reinhart, K. & International Forum of Acute Care Trialists. (2016). Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med* **193**, 259-72.
- [5] Cohen, J. (2002). The immunopathogenesis of sepsis. *Nature* **420**, 885-91.
- [6] Bhatia, M. & Moochhala, S. (2004). Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol* **202**, 145-56.
- [7] Russell, J. A. (2006). Management of sepsis. *N Engl J Med* **355**, 1699-713.
- [8] Wiersinga, W. J., Leopold, S. J., Cranendonk, D. R. & van der Poll, T. (2014). Host innate immune responses to sepsis. *Virulence* **5**, 36-44.
- [9] Kaprelyants, A. S., Gottschal, J. C. & Kell, D. B. (1993). Dormancy in non-sporulating bacteria. *FEMS Microbiol. Rev.* **10**, 271-286.
- [10] Kell, D. B., Kaprelyants, A. S., Weichart, D. H., Harwood, C. L. & Barer, M. R. (1998). Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. *Antonie van Leeuwenhoek* **73**, 169-187.
- [11] Lewis, K. (2007). Persister cells, dormancy and infectious disease. *Nat Rev Microbiol* **5**, 48-56.
- [12] Buerger, S., Spoering, A., Gavrish, E., Leslin, C., Ling, L. & Epstein, S. S. (2012). Microbial scout hypothesis, stochastic exit from dormancy, and the nature of slow growers. *Appl Environ Microbiol* **78**, 3221-8.
- [13] Kell, D. B., Potgieter, M. & Pretorius, E. (2015). Individuality, phenotypic differentiation, dormancy and 'persistence' in culturable bacterial systems: commonalities shared by environmental, laboratory, and clinical microbiology. *F1000Research* **4**, 179.
- [14] Lew, W. Y. W., Bayna, E., Molle, E. D., Dalton, N. D., Lai, N. C., Bhargava, V., Mendiola, V., Clopton, P. & Tang, T. (2013). Recurrent exposure to subclinical lipopolysaccharide increases mortality and induces cardiac fibrosis in mice. *PLoS One* **8**, e61057.
- [15] Morath, S., Geyer, A. & Hartung, T. (2001). Structure-function relationship of cytokine induction by lipoteichoic acid from *Staphylococcus aureus*. *J Exp Med* **193**, 393-7.
- [16] Prins, J. M., van Deventer, S. J. H., Kuijper, E. J. & Speelman, P. (1994). Clinical relevance of antibiotic-induced endotoxin release. *Antimicrob Agents Chemother* **38**, 1211-8.
- [17] Kirikae, T., Nakano, M. & Morrison, D. C. (1997). Antibiotic-induced endotoxin release from bacteria and its clinical significance. *Microbiol Immunol* **41**, 285-94.
- [18] Holzheimer, R. G. (2001). Antibiotic induced endotoxin release and clinical sepsis: a review. *J Chemother* **13 Spec No 1**, 159-72.

- [19] Lepper, P. M., Held, T. K., Schneider, E. M., Bölke, E., Gerlach, H. & Trautmann, M. (2002). Clinical implications of antibiotic-induced endotoxin release in septic shock. *Intensive Care Med* **28**, 824-33.
- [20] Levy, M. M., Fink, M. P., Marshall, J. C., Abraham, E., Angus, D., Cook, D., Cohen, J., Opal, S. M., Vincent, J. L., Ramsay, G. & Sccm/Esicm/Accp/Ats/Sis. (2003). 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* **31**, 1250-6.
- [21] Bozza, F. A., Salluh, J. I., Japiassu, A. M., Soares, M., Assis, E. F., Gomes, R. N., Bozza, M. T., Castro-Faria-Neto, H. C. & Bozza, P. T. (2007). Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. *Crit Care* **11**, R49.
- [22] D'Elia, R. V., Harrison, K., Oyston, P. C., Lukaszewski, R. A. & Clark, G. C. (2013). Targeting the "cytokine storm" for therapeutic benefit. *Clin Vaccine Immunol* **20**, 319-27.
- [23] Harrison, C. (2010). Sepsis: calming the cytokine storm. *Nat Rev Drug Discov* **9**, 360-1.
- [24] Oldstone, M. B. A. & Rosen, H. (2014). Cytokine storm plays a direct role in the morbidity and mortality from influenza virus infection and is chemically treatable with a single sphingosine-1-phosphate agonist molecule. *Curr Top Microbiol Immunol* **378**, 129-47.
- [25] Tisoncik, J. R., Korth, M. J., Simmons, C. P., Farrar, J., Martin, T. R. & Katze, M. G. (2012). Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* **76**, 16-32.
- [26] Wang, H. & Ma, S. (2008). The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. *Am J Emerg Med* **26**, 711-5.
- [27] Weigand, M. A., Hörner, C., Bardenheuer, H. J. & Bouchon, A. (2004). The systemic inflammatory response syndrome. *Best Pract Res Clin Anaesthesiol* **18**, 455-75.
- [28] Matsuda, N. & Hattori, Y. (2006). Systemic inflammatory response syndrome (SIRS): molecular pathophysiology and gene therapy. *J Pharmacol Sci* **101**, 189-98.
- [29] Ratzinger, F., Schuardt, M., Eichbichler, K., Tsirkinidou, I., Bauer, M., Haslacher, H., Mitteregger, D., Binder, M. & Burgmann, H. (2013). Utility of sepsis biomarkers and the infection probability score to discriminate sepsis and systemic inflammatory response syndrome in standard care patients. *PLoS One* **8**, e82946.
- [30] Reichsoellner, M., Raggam, R. B., Wagner, J., Krause, R. & Hoenigl, M. (2014). Clinical evaluation of multiple inflammation biomarkers for diagnosis and prognosis in patients with systemic inflammatory response syndrome. *J Clin Microbiol* **52**, 4063-4066.
- [31] Dunne, W. M., Jr. (2015). Laboratory diagnosis of sepsis? No SIRS, not just yet. *J Clin Microbiol* **53**, 2404-2409.
- [32] Stubljär, D. & Skvarca, M. (2015). Effective Strategies for Diagnosis of Systemic Inflammatory Response Syndrome (SIRS) due to Bacterial Infection in Surgical Patients. *Infect Disord Drug Targets* **15**, 53-56.
- [33] Laster, S. M., Wood, J. G. & Gooding, L. R. (1988). Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. *J Immunol* **141**, 2629-34.
- [34] Sridharan, H. & Upton, J. W. (2014). Programmed necrosis in microbial pathogenesis. *Trends Microbiol* **22**, 199-207.
- [35] Brown, K. A., Brain, S. D., Pearson, J. D., Edgeworth, J. D., Lewis, S. M. & Treacher, D. F. (2006). Neutrophils in development of multiple organ failure in sepsis. *Lancet* **368**, 157-69.
- [36] Johnson, D. & Mayers, I. (2001). Multiple organ dysfunction syndrome: a narrative review. *Can J Anaesth* **48**, 502-9.
- [37] Gando, S. (2010). Microvascular thrombosis and multiple organ dysfunction syndrome. *Crit Care Med* **38**, S35-S42.
- [38] Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J. D., Cooper-Smith, C. M., Hotchkiss, R. S., Levy, M. M., Marshall, J. C., Martin, G. S., Opal, S. M., Rubenfeld, G. D., van der Poll, T., Vincent, J. L. &

- Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **315**, 801-10.
- [39] Vincent, J. L., Moreno, R., Takala, J., Willatts, S., De Mendonca, A., Bruining, H., Reinhart, C. K., Suter, P. M. & Thijs, L. G. (1996). The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* **22**, 707-10.
- [40] Satran, R. & Almog, Y. (2003). The coagulopathy of sepsis: pathophysiology and management. *Isr Med Assoc J* **5**, 516-20.
- [41] Kinasewitz, G. T., Yan, S. B., Basson, B., Comp, P., Russell, J. A., Cariou, A., Um, S. L., Utterback, B., Laterre, P. F., Dhainaut, J. F. & Prowess Sepsis Study Group. (2004). Universal changes in biomarkers of coagulation and inflammation occur in patients with severe sepsis, regardless of causative micro-organism [ISRCTN74215569]. *Crit Care* **8**, R82-90.
- [42] Iba, T., Gando, S., Murata, A., Kushimoto, S., Saitoh, D., Eguchi, Y., Ohtomo, Y., Okamoto, K., Koseki, K., Mayumi, T., Ikeda, T., Ishikura, H., Ueyama, M., Ogura, Y., Endo, S. & Shimazaki, S. (2007). Predicting the severity of systemic inflammatory response syndrome (SIRS)-associated coagulopathy with hemostatic molecular markers and vascular endothelial injury markers. *J Trauma* **63**, 1093-8.
- [43] Ogura, H., Gando, S., Iba, T., Eguchi, Y., Ohtomo, Y., Okamoto, K., Koseki, K., Mayumi, T., Murata, A., Ikeda, T., Ishikura, H., Ueyama, M., Kushimoto, S., Saitoh, D., Endo, S. & Shimazaki, S. (2007). SIRS-associated coagulopathy and organ dysfunction in critically ill patients with thrombocytopenia. *Shock* **28**, 411-7.
- [44] Gando, S. (2013). Role of fibrinolysis in sepsis. *Semin Thromb Hemost* **39**, 392-9.
- [45] Hoppensteadt, D., Tsuruta, K., Cunanan, J., Hirman, J., Kaul, I., Osawa, Y. & Fareed, J. (2014). Thrombin generation mediators and markers in sepsis-associated coagulopathy and their modulation by recombinant thrombomodulin. *Clin Appl Thromb Hemost* **20**, 129-35.
- [46] Ostrowski, S. R., Berg, R. M. G., Windeløv, N. A., Meyer, M. A. S., Plovsing, R. R., Møller, K. & Johansson, P. I. (2013). Coagulopathy, catecholamines, and biomarkers of endothelial damage in experimental human endotoxemia and in patients with severe sepsis: a prospective study. *J Crit Care* **28**, 586-96.
- [47] Saracco, P., Vitale, P., Scolfaro, C., Pollio, B., Pagliarino, M. & Timeus, F. (2011). The coagulopathy in sepsis: significance and implications for treatment. *Pediatr Rep* **3**, e30.
- [48] Semeraro, N., Ammollo, C. T., Semeraro, F. & Colucci, M. (2015). Coagulopathy of Acute Sepsis. *Semin Thromb Hemost* **41**, 650-8.
- [49] Simmons, J. & Pittet, J. F. (2015). The coagulopathy of acute sepsis. *Curr Opin Anaesthesiol* **28**, 227-36.
- [50] Kell, D. B. (2009). Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med Genom* **2**, 2
- [51] Kell, D. B. (2010). Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Arch Toxicol* **577**, 825-889. .
- [52] Kell, D. B. & Pretorius, E. (2014). Serum ferritin is an important disease marker, and is mainly a leakage product from damaged cells. *Metallomics* **6**, 748-773.
- [53] Pretorius, E. & Kell, D. B. (2014). Diagnostic morphology: biophysical indicators for iron-driven inflammatory diseases. *Integrative Biol* **6**, 486-510.
- [54] Kell, D. B. & Pretorius, E. (2015). The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen). *Integr Biol* **7**, 24-52.

- [55] Kell, D. B. & Pretorius, E. (2015). On the translocation of bacteria and their lipopolysaccharides between blood and peripheral locations in chronic, inflammatory diseases: the central roles of LPS and LPS-induced cell death *Integr Biol* **7**, 1339-1377.
- [56] Potgieter, M., Bester, J., Kell, D. B. & Pretorius, E. (2015). The dormant blood microbiome in chronic, inflammatory diseases. *FEMS Microbiol Rev* **39**, 567-591.
- [57] Kell, D. B. & Kenny, L. C. (2016). A dormant microbial component in the development of pre-eclampsia. BioRxiv preprint. . *bioRxiv*, 057356.
- [58] Marshall, B. J. & Warren, J. R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* **1**, 1311-1315.
- [59] Itzhaki, R. F., Lathe, R., Balin, B. J., Ball, M. J., Braak, H., Bearer, E. L., Bullido, M. J., Carter, C., Clerici, M., Cosby, S. L., Del Tredici, K., Field, H., Fulop, T., Grassi, C., Griffin, W. S. T., Haas, J., Hudson, A. P., Kamer, A., Kell, D. B., Licastro, F., Letenneur, L., Lövheim, H., Mancuso, R., Miklossy, J., Otth, C., Palamara, A. T., Perry, G., Preston, C., Pretorius, E., Strandberg, T., Tabet, N., Taylor-Robinson, S. D. & Whittum-Hudson, J. A. (2016). Microbes and Alzheimer's Disease. *J Alzheimers Dis* **51**, 979-984.
- [60] Weisel, J. W. (2005). Fibrinogen and fibrin. *Adv Protein Chem* **70**, 247-99.
- [61] Cilia La Corte, A. L., Philippou, H. & Ariëns, R. A. S. (2011). Role of fibrin structure in thrombosis and vascular disease. *Adv Protein Chem Struct Biol* **83**, 75-127.
- [62] Undas, A. & Ariëns, R. A. S. (2011). Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. *Arterioscler Thromb Vasc Biol* **31**, e88-99.
- [63] Undas, A., Nowakowski, T., Cieśla-Dul, M. & Sadowski, J. (2011). Abnormal plasma fibrin clot characteristics are associated with worse clinical outcome in patients with peripheral arterial disease and thromboangiitis obliterans. *Atherosclerosis* **215**, 481-6.
- [64] Wolberg, A. S. (2012). Determinants of fibrin formation, structure, and function. *Curr Opin Hematol* **19**, 349-56.
- [65] Undas, A. (2014). Fibrin clot properties and their modulation in thrombotic disorders. *Thromb Haemost* **112**, 32-42.
- [66] Pretorius, E., Vermeulen, N., Bester, J., Lipinski, B. & Kell, D. B. (2013). A novel method for assessing the role of iron and its functional chelation in fibrin fibril formation: the use of scanning electron microscopy. *Toxicol Mech Methods* **23**, 352-359.
- [67] Pretorius, E., Bester, J., Vermeulen, N., Lipinski, B., Gericke, G. S. & Kell, D. B. (2014). Profound morphological changes in the erythrocytes and fibrin networks of patients with hemochromatosis or with hyperferritinemia, and their normalization by iron chelators and other agents. *PLoS One* **9**, e85271.
- [68] Pretorius, E., Bester, J., Vermeulen, N., Alummoottil, S., Soma, P., Buys, A. V. & Kell, D. B. (2015). Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics. *Cardiovasc Diabetol* **13**, 30.
- [69] Pretorius, E., Windberger, U. B., Oberholzer, H. M. & Auer, R. E. (2010). Comparative ultrastructure of fibrin networks of a dog after thrombotic ischaemic stroke. *Onderstepoort J Vet Res* **77**, E1-4.
- [70] Pretorius, E., Swanepoel, A. C., Oberholzer, H. M., van der Spuy, W. J., Duim, W. & Wessels, P. F. (2011). A descriptive investigation of the ultrastructure of fibrin networks in thromboembolic ischemic stroke. *J Thromb Thrombolysis* **31**, 507-13.
- [71] Pretorius, E., Steyn, H., Engelbrecht, M., Swanepoel, A. C. & Oberholzer, H. M. (2011). Differences in fibrin fiber diameters in healthy individuals and thromboembolic ischemic stroke patients. *Blood Coagul Fibrinolysis* **22**, 696-700.
- [72] Pretorius, E., Oberholzer, H. M., van der Spuy, W. J., Swanepoel, A. C. & Soma, P. (2011). Qualitative scanning electron microscopy analysis of fibrin networks and platelet abnormalities in diabetes. *Blood Coagul Fibrinol* **22**, 463-7.

- [73] Bester, J., Soma, P., Kell, D. B. & Pretorius, E. (2015). Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). *Oncotarget Gerontology* **6**, 35284-35303.
- [74] Lipinski, B. & Pretorius, W. (2014). Iron-induced fibrin formation may explain vascular pathology in Alzheimer's disease. *Folia Neuropathol* **52**, 205.
- [75] Pretorius, E., Bester, J. & Kell, D. B. (2016). A bacterial component to Alzheimer-type dementia seen via a systems biology approach that links iron dysregulation and inflammagen shedding to disease *J Alzheimers Dis*, in press.
- [76] Lipinski, B. & Pretorius, E. (2012). Novel pathway of iron-induced blood coagulation: implications for diabetes mellitus and its complications. *Pol Arch Med Wewn* **122**, 115-22.
- [77] Lipinski, B. & Pretorius, E. (2013). Iron-induced fibrin in cardiovascular disease. *Curr neurovasc res* **10**, 269-274.
- [78] Pretorius, E., Vermeulen, N., Bester, J. & Lipinski, B. (2013). Novel use of scanning electron microscopy for detection of iron-induced morphological changes in human blood. *Microsc Res Tech* **76**, 268-271.
- [79] Asakura, H. (2014). Classifying types of disseminated intravascular coagulation: clinical and animal models. *J Intensive Care* **2**, 20.
- [80] Duburcq, T., Tournoys, A., Gnemmi, V., Hubert, T., Gmyr, V., Pattou, F. & Jourdain, M. (2015). Impact of Obesity on Endotoxin-Induced Disseminated Intravascular Coagulation. *Shock* **44**, 341-7.
- [81] Bick, R. L. (2002). Disseminated intravascular coagulation: a review of etiology, pathophysiology, diagnosis, and management: guidelines for care. *Clin Appl Thromb Hemost* **8**, 1-31.
- [82] Kaneko, T. & Wada, H. (2011). Diagnostic criteria and laboratory tests for disseminated intravascular coagulation. *J Clin Exp Hematop* **51**, 67-76.
- [83] Levi, M. (2010). The coagulant response in sepsis and inflammation. *Hamostaseologie* **30**, 10-2, 14-6.
- [84] Paulus, P., Jennewein, C. & Zacharowski, K. (2011). Biomarkers of endothelial dysfunction: can they help us deciphering systemic inflammation and sepsis? *Biomarkers* **16 Suppl 1**, S11-21.
- [85] Khemani, R. G., Bart, R. D., Alonzo, T. A., Hatzakis, G., Hallam, D. & Newth, C. J. L. (2009). Disseminated intravascular coagulation score is associated with mortality for children with shock. *Intensive Care Med* **35**, 327-33.
- [86] Levi, M. & van der Poll, T. (2013). Disseminated intravascular coagulation: a review for the internist. *Int Emerg Med* **8**, 23-32.
- [87] Thachil, J. & Toh, C. H. (2012). Current concepts in the management of disseminated intravascular coagulation. *Thromb Res* **129 Suppl 1**, S54-9.
- [88] Wada, H., Matsumoto, T., Yamashita, Y. & Hatada, T. (2014). Disseminated intravascular coagulation: testing and diagnosis. *Clin Chim Acta* **436**, 130-4.
- [89] Wu, L. C., Lin, X. & Sun, H. (2012). Tanshinone IIA protects rabbits against LPS-induced disseminated intravascular coagulation (DIC). *Acta Pharmacol Sin* **33**, 1254-9.
- [90] Wu, Z., Li, J. N., Bai, Z. Q. & Lin, X. (2014). Antagonism by salvianolic acid B of lipopolysaccharide-induced disseminated intravascular coagulation in rabbits. *Clin Exp Pharmacol Physiol* **41**, 502-8.
- [91] Yu, P. X., Zhou, Q. J., Zhu, W. W., Wu, Y. H., Wu, L. C., Lin, X., Chen, M. H. & Qiu, B. T. (2013). Effects of quercetin on LPS-induced disseminated intravascular coagulation (DIC) in rabbits. *Thromb Res* **131**, e270-3.
- [92] Nguyen, T. C., Gushiken, F., Correa, J. I., Dong, J. F., Dasgupta, S. K., Thiagarajan, P. & Cruz, M. A. (2015). A recombinant fragment of von Willebrand factor reduces fibrin-rich microthrombi formation in mice with endotoxemia. *Thromb Res* **135**, 1025-30.
- [93] Zeerleder, S., Hack, C. E. & Wuillemin, W. A. (2005). Disseminated intravascular coagulation in sepsis. *Chest* **128**, 2864-75.

- [94] Gando, S., Saitoh, D., Ogura, H., Mayumi, T., Koseki, K., Ikeda, T., Ishikura, H., Iba, T., Ueyama, M., Eguchi, Y., Ohtomo, Y., Okamoto, K., Kushimoto, S., Endo, S., Shimazaki, S. & Japanese Association for Acute Medicine Disseminated Intravascular Coagulation Study, G. (2008). Natural history of disseminated intravascular coagulation diagnosed based on the newly established diagnostic criteria for critically ill patients: results of a multicenter, prospective survey. *Crit Care Med* **36**, 145-50.
- [95] Semeraro, N., Ammollo, C. T., Semeraro, F. & Colucci, M. (2010). Sepsis-associated disseminated intravascular coagulation and thromboembolic disease. *Mediterr J Hematol Infect Dis* **2**, e2010024.
- [96] Gando, S., Meziani, F. & Levi, M. (2016). What's new in the diagnostic criteria of disseminated intravascular coagulation? *Intensive Care Med* **42**, 1062-4.
- [97] Cunningham, F. G. & Nelson, D. B. (2015). Disseminated Intravascular Coagulation Syndromes in Obstetrics. *Obstet Gynecol* **126**, 999-1011.
- [98] Anfinsen, C. B., Haber, E., Sela, M. & White, F. H. (1961). The kinetics of formation of native ribonuclease during oxidation of the reduced polypeptide chain. *Proc. Natl. acad. Sci.* **47**, 1309-1314.
- [99] Anfinsen, C. B. (1973). Principles that govern the folding of protein chains. *Science* **181**, 223-230.
- [100] Cohen, F. E. & Prusiner, S. B. (1998). Pathologic conformations of prion proteins. *Annu Rev Biochem* **67**, 793-819.
- [101] Harrison, P. M., Chan, H. S., Prusiner, S. B. & Cohen, F. E. (1999). Thermodynamics of model prions and its implications for the problem of prion protein folding. *J Mol Biol* **286**, 593-606.
- [102] Collinge, J. & Clarke, A. R. (2007). A general model of prion strains and their pathogenicity. *Science* **318**, 930-6.
- [103] Aguzzi, A., Sigurdson, C. & Heikenwaelder, M. (2008). Molecular mechanisms of prion pathogenesis. *Annu Rev Pathol* **3**, 11-40.
- [104] Aguzzi, A. & Calella, A. M. (2009). Prions: protein aggregation and infectious diseases. *Physiol Rev* **89**, 1105-52.
- [105] Ashe, K. H. & Aguzzi, A. (2013). Prions, prionoids and pathogenic proteins in Alzheimer disease. *Prion* **7**, 55-9.
- [106] Watts, J. C., Condello, C., Stohr, J., Oehler, A., Lee, J., DeArmond, S. J., Lannfelt, L., Ingelsson, M., Giles, K. & Prusiner, S. B. (2014). Serial propagation of distinct strains of Abeta prions from Alzheimer's disease patients. *Proc Natl Acad Sci* **111**, 10323-8.
- [107] Woerman, A. L., Stöhr, J., Aoyagi, A., Rampersaud, R., Krejciova, Z., Watts, J. C., Ohyama, T., Patel, S., Widjaja, K., Oehler, A., Sanders, D. W., Diamond, M. I., Seeley, W. W., Middleton, L. T., Gentleman, S. M., Mordes, D. A., Südhof, T. C., Giles, K. & Prusiner, S. B. (2015). Propagation of prions causing synucleinopathies in cultured cells. *Proc Natl Acad Sci U S A* **112**, E4949-58.
- [108] Benson, M. D., Liepnieks, J., Uemichi, T., Wheeler, G. & Correa, R. (1993). Hereditary renal amyloidosis associated with a mutant fibrinogen alpha-chain. *Nat Genet* **3**, 252-5.
- [109] Hamidi Asl, L., Liepnieks, J. J., Uemichi, T., Rebibou, J. M., Justrabo, E., Droz, D., Mousson, C., Chalopin, J. M., Benson, M. D., Delpech, M. & Grateau, G. (1997). Renal amyloidosis with a frame shift mutation in fibrinogen alpha-chain gene producing a novel amyloid protein. *Blood* **90**, 4799-805.
- [110] Serpell, L. C., Benson, M., Liepnieks, J. J. & Fraser, P. E. (2007). Structural analyses of fibrinogen amyloid fibrils. *Amyloid* **14**, 199-203.
- [111] Gillmore, J. D., Lachmann, H. J., Rowczenio, D., Gilbertson, J. A., Zeng, C. H., Liu, Z. H., Li, L. S., Wechalekar, A. & Hawkins, P. N. (2009). Diagnosis, pathogenesis, treatment, and prognosis of hereditary fibrinogen A alpha-chain amyloidosis. *J Am Soc Nephrol* **20**, 444-51.
- [112] Picken, M. M. (2010). Fibrinogen amyloidosis: the clot thickens! *Blood* **115**, 2985-6.
- [113] Stangou, A. J., Banner, N. R., Hendry, B. M., Rela, M., Portmann, B., Wendon, J., Monaghan, M., Mccarthy, P., Buxton-Thomas, M., Mathias, C. J., Liepnieks, J. J., O'Grady, J., Heaton, N. D. &

- Benson, M. D. (2010). Hereditary fibrinogen A alpha-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. *Blood* **115**, 2998-3007.
- [114] Haidinger, M., Werzowa, J., Kain, R., Antlanger, M., Hecking, M., Pfaffenberger, S., Mascherbauer, J., Gremmel, T., Gilbertson, J. A., Rowczenio, D., Weichhart, T., Kopecky, C., Hörl, W. H., Hawkins, P. N. & Säemann, M. D. (2013). Hereditary amyloidosis caused by R554L fibrinogen Aalpha-chain mutation in a Spanish family and review of the literature. *Amyloid* **20**, 72-9.
- [115] Zhmurov, A., Brown, A. E., Litvinov, R. I., Dima, R. I., Weisel, J. W. & Barsegov, V. (2011). Mechanism of fibrin(ogen) forced unfolding. *Structure* **19**, 1615-24.
- [116] Litvinov, R. I., Faizullin, D. A., Zuev, Y. F. & Weisel, J. W. (2012). The alpha-helix to beta-sheet transition in stretched and compressed hydrated fibrin clots. *Biophys J* **103**, 1020-7.
- [117] Zhmurov, A., Kononova, O., Litvinov, R. I., Dima, R. I., Barsegov, V. & Weisel, J. W. (2012). Mechanical transition from alpha-helical coiled coils to beta-sheets in fibrin(ogen). *J Am Chem Soc* **134**, 20396-402.
- [118] Kreplak, L., Doucet, J., Dumas, P. & Briki, F. (2004). New aspects of the alpha-helix to beta-sheet transition in stretched hard alpha-keratin fibers. *Biophys J* **87**, 640-7.
- [119] Guthold, M., Liu, W., Sparks, E. A., Jawerth, L. M., Peng, L., Falvo, M., Superfine, R., Hantgan, R. & Lord, S. T. (2007). A comparison of the mechanical and structural properties of fibrin fibers with other protein fibers. *Cell Biochem Biophys* **49**, 165-81.
- [120] Liu, W., Carlisle, C. R., Sparks, E. A. & Guthold, M. (2010). The mechanical properties of single fibrin fibers. *J Thromb Haemost* **8**, 1030-6.
- [121] Miserez, A. & Guerette, P. A. (2013). Phase transition-induced elasticity of alpha-helical bioelastomeric fibres and networks. *Chem Soc Rev* **42**, 1973-95.
- [122] Pretorius, E., Mbotwe, S., Bester, J., Robinson, C. & Kell, D. B. (2016). Acute induction of anomalous blood clotting by highly substoichiometric levels of bacterial lipopolysaccharide. *J R Soc Interface*, submitted.
- [123] Kell, D. B. & Pretorius, E. (2016). Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. bioRxiv preprint. *bioRxiv*, 054734.
- [124] Weisel, J. W. (2007). Structure of fibrin: impact on clot stability. *J Thromb Haemost* **5 Suppl 1**, 116-24.
- [125] Wolberg, A. S. (2007). Thrombin generation and fibrin clot structure. *Blood Rev* **21**, 131-42.
- [126] Biancalana, M., Makabe, K., Koide, A. & Koide, S. (2009). Molecular mechanism of thioflavin-T binding to the surface of beta-rich peptide self-assemblies. *J Mol Biol* **385**, 1052-63.
- [127] Biancalana, M. & Koide, S. (2010). Molecular mechanism of Thioflavin-T binding to amyloid fibrils. *Biochim Biophys Acta* **1804**, 1405-12.
- [128] Groenning, M. (2010). Binding mode of Thioflavin T and other molecular probes in the context of amyloid fibrils-current status. *J Chem Biol* **3**, 1-18.
- [129] Kuznetsova, I. M., Sulatskaya, A. I., Uversky, V. N. & Turoverov, K. K. (2012). Analyzing thioflavin T binding to amyloid fibrils by an equilibrium microdialysis-based technique. *PLoS One* **7**, e30724.
- [130] Kuznetsova, I. M., Sulatskaya, A. I., Uversky, V. N. & Turoverov, K. K. (2012). A new trend in the experimental methodology for the analysis of the thioflavin T binding to amyloid fibrils. *Mol Neurobiol* **45**, 488-98.
- [131] Kuznetsova, I. M., Sulatskaya, A. I., Maskevich, A. A., Uversky, V. N. & Turoverov, K. K. (2016). High Fluorescence Anisotropy of Thioflavin T in Aqueous Solution Resulting from Its Molecular Rotor Nature. *Anal Chem* **88**, 718-24.
- [132] Lindberg, D. J., Wranne, M. S., Gilbert Gatty, M., Westerlund, F. & Esbjörner, E. K. (2015). Steady-state and time-resolved Thioflavin-T fluorescence can report on morphological

- differences in amyloid fibrils formed by Abeta(1-40) and Abeta(1-42). *Biochem Biophys Res Commun* **458**, 418-23.
- [133] Sulatskaya, A. I., Kuznetsova, I. M. & Turoverov, K. K. (2012). Interaction of thioflavin T with amyloid fibrils: fluorescence quantum yield of bound dye. *J Phys Chem B* **116**, 2538-44.
- [134] Younan, N. D. & Viles, J. H. (2015). A Comparison of Three Fluorophores for the Detection of Amyloid Fibers and Prefibrillar Oligomeric Assemblies. ThT (Thioflavin T); ANS (1-Anilinonaphthalene-8-sulfonic Acid); and bisANS (4,4'-Dianilino-1,1'-binaphthyl-5,5'-disulfonic Acid). *Biochemistry* **54**, 4297-306.
- [135] Zhang, X. & Ran, C. (2013). Dual Functional Small Molecule Probes as Fluorophore and Ligand for Misfolding Proteins. *Curr Org Chem* **17**.
- [136] Akassoglou, K., Adams, R. A., Bauer, J., Mercado, P., Tseveleki, V., Lassmann, H., Probert, L. & Strickland, S. (2004). Fibrin depletion decreases inflammation and delays the onset of demyelination in a tumor necrosis factor transgenic mouse model for multiple sclerosis. *Proc Natl Acad Sci U S A* **101**, 6698-703.
- [137] Levi, M., van der Poll, T. & Buller, H. R. (2004). Bidirectional relation between inflammation and coagulation. *Circulation* **109**, 2698-704.
- [138] Flick, M. J., Lajeunesse, C. M., Talmage, K. E., Witte, D. P., Palumbo, J. S., Pinkerton, M. D., Thornton, S. & Degen, J. L. (2007). Fibrin(ogen) exacerbates inflammatory joint disease through a mechanism linked to the integrin alphaMbeta2 binding motif. *J Clin Invest* **117**, 3224-35.
- [139] Jennewein, C., Tran, N., Paulus, P., Ellinghaus, P., Eble, J. A. & Zacharowski, K. (2011). Novel aspects of fibrin(ogen) fragments during inflammation. *Mol Med* **17**, 568-73.
- [140] Jennewein, C., Paulus, P. & Zacharowski, K. (2011). Linking inflammation and coagulation: novel drug targets to treat organ ischemia. *Curr Opin Anaesthesiol* **24**, 375-80.
- [141] Schuliga, M. (2015). The inflammatory actions of coagulant and fibrinolytic proteases in disease. *Mediators Inflamm* **2015**, 437695.
- [142] Lin, I. H., Miller, D. S., Bertics, P. J., Murphy, C. J., de Pablo, J. J. & Abbott, N. L. (2011). Endotoxin-induced structural transformations in liquid crystalline droplets. *Science* **332**, 1297-300.
- [143] Miller, D. S. & Abbott, N. L. (2013). Influence of droplet size, pH and ionic strength on endotoxin-triggered ordering transitions in liquid crystalline droplets. *Soft Matter* **9**, 374-382.
- [144] Carter, M. C. D., Miller, D. S., Jennings, J., Wang, X. G., Mahanthappa, M. K., Abbott, N. L. & Lynn, D. M. (2015). Synthetic Mimics of Bacterial Lipid A Trigger Optical Transitions in Liquid Crystal Microdroplets at Ultra low Picogram-per-Milliliter Concentrations. *Langmuir* **31**, 12850-12855.
- [145] Ahmed, M., Davis, J., Aucoin, D., Sato, T., Ahuja, S., Aimoto, S., Elliott, J. I., Van Nostrand, W. E. & Smith, S. O. (2010). Structural conversion of neurotoxic amyloid-beta₁₋₄₂ oligomers to fibrils. *Nat Struct Mol Biol* **17**, 561-7.
- [146] Hefti, F., Goure, W. F., Jerecic, J., Iverson, K. S., Walicke, P. A. & Krafft, G. A. (2013). The case for soluble Abeta oligomers as a drug target in Alzheimer's disease. *Trends Pharmacol Sci* **34**, 261-6.
- [147] Kaye, R. & Lasagna-Reeves, C. A. (2013). Molecular mechanisms of amyloid oligomers toxicity. *J Alzheimers Dis* **33** Suppl 1, S67-78.
- [148] Liu, B., Moloney, A., Meehan, S., Morris, K., Thomas, S. E., Serpell, L. C., Hider, R., Marciniak, S. J., Lomas, D. A. & Crowther, D. C. (2011). Iron promotes the toxicity of amyloid beta peptide by impeding its ordered aggregation. *J Biol Chem* **286**, 4248-56.
- [149] Meyer-Luehmann, M., Spires-Jones, T. L., Prada, C., Garcia-Alloza, M., de Calignon, A., Rozkalne, A., Koenigsnecht-Talboo, J., Holtzman, D. M., Bacskai, B. J. & Hyman, B. T. (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature* **451**, 720-4.

- [150] Minter, M. R., Taylor, J. M. & Crack, P. J. (2016). The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. *J Neurochem* **136**, 457-74.
- [151] Miranda, S., Opazo, C., Larrondo, L. F., Munoz, F. J., Ruiz, F., Leighton, F. & Inestrosa, N. C. (2000). The role of oxidative stress in the toxicity induced by amyloid beta-peptide in Alzheimer's disease. *Progr Neurobiol* **62**, 633-648.
- [152] Rival, T., Page, R. M., Chandraratna, D. S., Sendall, T. J., Ryder, E., Liu, B., Lewis, H., Rosahl, T., Hider, R., Camargo, L. M., Shearman, M. S., Crowther, D. C. & Lomas, D. A. (2009). Fenton chemistry and oxidative stress mediate the toxicity of the beta-amyloid peptide in a *Drosophila* model of Alzheimer's disease. *Eur J Neurosci* **29**, 1335-1347.
- [153] Sengupta, U., Nilson, A. N. & Kaye, R. (2016). The Role of Amyloid-beta Oligomers in Toxicity, Propagation, and Immunotherapy. *EBioMedicine* **6**, 42-9.
- [154] Uversky, V. N. (2010). Mysterious oligomerization of the amyloidogenic proteins. *FEBS J* **277**, 2940-53.
- [155] Barton, C. H., Vaziri, N. D., Gordon, S. & Tilles, S. (1984). Renal pathology in end-stage renal disease associated with paraplegia. *Paraplegia* **22**, 31-41.
- [156] Jadoul, M., Garbar, C., Noel, H., Sennesael, J., Vanholder, R., Bernaert, P., Rorive, G., Hanique, G. & van Ypersele de Strihou, C. (1997). Histological prevalence of beta 2-microglobulin amyloidosis in hemodialysis: a prospective post-mortem study. *Kidney Int* **51**, 1928-32.
- [157] Ben-Dov, I. Z., Pizov, G., Ben-Chetrit, E., Rubinger, D. & Or, R. (2009). Fatal nephrotic syndrome complicating allogeneic stem cell transplantation: a case report. *Nephrol Dial Transplant* **24**, 2946-9.
- [158] Lachmann, H. J. (2010). Secondary, AA, Amyloidosis. *Amyloidosis: Diagnosis and Treatment*, 179-189.
- [159] Lucas, S. (2012). The Autopsy Pathology of Sepsis-Related Death, Severe Sepsis and Septic Shock - Understanding a Serious Killer (ed. R. Fernandez), pp. <http://www.intechopen.com/books/severe-sepsis-and-septic-shock-understanding-a-serious-killer/theautopsy-pathology-of-sepsis-and-septic-shock>. InTech Open, Rijeka.
- [160] Díez, R., Madero, M., Gamba, G., Soriano, J. & Soto, V. (2014). Renal AA Amyloidosis in Patients with Type 2 Diabetes Mellitus. *Nephron Extra* **4**, 119-26.
- [161] Helmerhorst, E. J., Maaskant, J. J. & Appelmelk, B. J. (1998). Anti-lipid A monoclonal antibody centoxin (HA-1A) binds to a wide variety of hydrophobic ligands. *Infect Immun* **66**, 870-3.
- [162] Marks, L. (2012). The birth pangs of monoclonal antibody therapeutics: the failure and legacy of Centoxin. *MAbs* **4**, 403-12.
- [163] Murphy, S. T. & Bellamy, M. C. (2013). The quest for the magic bullet: Centoxin, Drotrecogin Alfa and lessons not learned. *Trends AnaesTh Crit Care* **3**, 316-319.
- [164] Martí-Carvajal, A. J., Solà, I., Gluud, C., Lathyris, D. & Cardona, A. F. (2012). Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients. *Cochrane Database Syst Rev* **12**, CD004388.
- [165] Lai, P. S., Matteau, A., Idriss, A., Hawes, J. C., Ranieri, V. & Thompson, B. T. (2013). An updated meta-analysis to understand the variable efficacy of drotrecogin alfa (activated) in severe sepsis and septic shock. *Minerva Anesthesiol* **79**, 33-43.
- [166] Nadel, S., Goldstein, B., Williams, M. D., Dalton, H., Peters, M., Macias, W. L., Abd-Allah, S. A., Levy, H., Angle, R., Wang, D., Sundin, D. P., Giroir, B., Sepsis, R. E. s. & Organ dysfunction in children: a gLocal perspective study group. (2007). Drotrecogin alfa (activated) in children with severe sepsis: a multicentre phase III randomised controlled trial. *Lancet* **369**, 836-43.
- [167] Ranieri, V. M., Thompson, B. T., Barie, P. S., Dhainaut, J. F., Douglas, I. S., Finfer, S., Gardlund, B., Marshall, J. C., Rhodes, A., Artigas, A., Payen, D., Tenhunen, J., Al-Khalidi, H. R., Thompson, V., Janes, J., Macias, W. L., Vangerow, B., Williams, M. D. & Group, P.-S. S. (2012). Drotrecogin alfa (activated) in adults with septic shock. *N Engl J Med* **366**, 2055-64.
- [168] Minnich, D. J. & Moldawer, L. L. (2004). Anti-cytokine and anti-inflammatory therapies for the treatment of severe sepsis: progress and pitfalls. *Proc Nutr Soc* **63**, 437-41.

- [169] Aziz, M., Jacob, A., Yang, W. L., Matsuda, A. & Wang, P. (2013). Current trends in inflammatory and immunomodulatory mediators in sepsis. *J Leukoc Biol* **93**, 329-42.
- [170] Kell, D. B. (2013). Finding novel pharmaceuticals in the systems biology era using multiple effective drug targets, phenotypic screening, and knowledge of transporters: where drug discovery went wrong and how to fix it. *FEBS J* **280**, 5957-5980.
- [171] Weinberg, E. D. (1984). Iron withholding: a defense against infection and neoplasia. *Physiol Rev* **64**, 65-102.
- [172] Xia, Y., Farah, N., Maxan, A., Zhou, J. & Lehmann, C. (2016). Therapeutic iron restriction in sepsis. *Med Hypotheses* **89**, 37-9.
- [173] Chapin, J. C. & Hajjar, K. A. (2015). Fibrinolysis and the control of blood coagulation. *Blood Rev* **29**, 17-24.
- [174] Cornet, A. D., Smit, E. G., Beishuizen, A. & Groeneveld, A. B. (2007). The role of heparin and allied compounds in the treatment of sepsis. *Thromb Haemost* **98**, 579-86.
- [175] Wang, C., Chi, C., Guo, L., Wang, X., Guo, L., Sun, J., Sun, B., Liu, S., Chang, X. & Li, E. (2014). Heparin therapy reduces 28-day mortality in adult severe sepsis patients: a systematic review and meta-analysis. *Crit Care* **18**, 563.
- [176] Zarychanski, R., Abou-Setta, A. M., Kanji, S., Turgeon, A. F., Kumar, A., Houston, D. S., Rimmer, E., Houston, B. L., McIntyre, L., Fox-Robichaud, A. E., Hebert, P., Cook, D. J., Fergusson, D. A. & Canadian Critical Care Trials, G. (2015). The efficacy and safety of heparin in patients with sepsis: a systematic review and metaanalysis. *Crit Care Med* **43**, 511-8.
- [177] van Roessel, S., van der Laan, A. M. & de Pont, A.-C. J. M. (2015). What is the best heparin to treat sepsis with? *Crit Care Med* **43**, e212-3.
- [178] Okamoto, K., Tamura, T. & Sawatsubashi, Y. (2016). Sepsis and disseminated intravascular coagulation. *J Intensive Care* **4**, 23.
- [179] Rosenberg, V. A., Buhimschi, I. A., Lockwood, C. J., Paidas, M. J., Dulay, A. T., Ramma, W., Abdel-Razeq, S. S., Zhao, G., Ahmad, S., Ahmed, A. & Buhimschi, C. S. (2011). Heparin elevates circulating soluble fms-like tyrosine kinase-1 immunoreactivity in pregnant women receiving anticoagulation therapy. *Circulation* **124**, 2543-53.
- [180] Iba, T., Hashiguchi, N., Nagaoka, I., Tabe, Y., Kadota, K. & Sato, K. (2015). Heparins attenuated histone-mediated cytotoxicity *in vitro* and improved the survival in a rat model of histone-induced organ dysfunction. *Intensive Care Med Exp* **3**, 36.
- [181] Chien, J. Y., Jerng, J. S., Yu, C. J. & Yang, P. C. (2005). Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Crit Care Med* **33**, 1688-93.
- [182] Levels, J. H. M., Geurts, P., Karlsson, H., Marée, R., Ljunggren, S., Fornander, L., Wehenkel, L., Lindahl, M., Stroes, E. S. G., Kuivenhoven, J. A. & Meijers, J. C. M. (2011). High-density lipoprotein proteome dynamics in human endotoxemia. *Proteome Sci* **9**, 34.
- [183] Catapano, A. L., Pirillo, A., Bonacina, F. & Norata, G. D. (2014). HDL in innate and adaptive immunity. *Cardiovasc Res* **103**, 372-83.
- [184] Bandeali, S. & Farmer, J. (2012). High-density lipoprotein and atherosclerosis: the role of antioxidant activity. *Curr Atheroscler Rep* **14**, 101-7.
- [185] Tabet, F. & Rye, K. A. (2009). High-density lipoproteins, inflammation and oxidative stress. *Clin Sci (Lond)* **116**, 87-98.
- [186] Guo, L., Ai, J., Zheng, Z., Howatt, D. A., Daugherty, A., Huang, B. & Li, X. A. (2013). High density lipoprotein protects against polymicrobe-induced sepsis in mice. *J Biol Chem* **288**, 17947-53.
- [187] Contreras-Duarte, S., Varas, P., Awad, F., Busso, D. & Rigotti, A. (2014). [Protective role of high density lipoproteins in sepsis: basic issues and clinical implications]. *Rev Chilena Infectol* **31**, 34-43.
- [188] Pirillo, A., Catapano, A. L. & Norata, G. D. (2015). HDL in infectious diseases and sepsis. *Handb Exp Pharmacol* **224**, 483-508.

- [189] Almog, Y., Shefer, A., Novack, V., Maimon, N., Barski, L., Eizinger, M., Friger, M., Zeller, L. & Danon, A. (2004). Prior statin therapy is associated with a decreased rate of severe sepsis. *Circulation* **110**, 880-5.
- [190] Dobesh, P. P. & Olsen, K. M. (2014). Statins role in the prevention and treatment of sepsis. *Pharmacol Res* **88**, 31-40.
- [191] Harvey, M. & Cave, G. (2014). Co-administration of phospholipid emulsion with first dose bacteriocidal antibiotic may retard progression of the sepsis response in gram negative septicaemia. *Med Hypotheses* **83**, 563-5.
- [192] Saito, H., Maruyama, I., Shimazaki, S., Yamamoto, Y., Aikawa, N., Ohno, R., Hirayama, A., Matsuda, T., Asakura, H., Nakashima, M. & Aoki, N. (2007). Efficacy and safety of recombinant human soluble thrombomodulin (ART-123) in disseminated intravascular coagulation: results of a phase III, randomized, double-blind clinical trial. *J Thromb Haemost* **5**, 31-41.
- [193] Levi, M. & Van Der Poll, T. (2013). Thrombomodulin in sepsis. *Minerva Anesthesiol* **79**, 294-8.
- [194] Mimuro, J., Takahashi, H., Kitajima, I., Tsuji, H., Eguchi, Y., Matsushita, T., Kuroda, T. & Sakata, Y. (2013). Impact of recombinant soluble thrombomodulin (thrombomodulin alfa) on disseminated intravascular coagulation. *Thromb Res* **131**, 436-43.
- [195] Vincent, J. L., Ramesh, M. K., Ernest, D., LaRosa, S. P., Pacht, J., Aikawa, N., Hoste, E., Levy, H., Hirman, J., Levi, M., Daga, M., Kutsogiannis, D. J., Crowther, M., Bernard, G. R., Devriendt, J., Puigserver, J. V., Blanzaco, D. U., Esmon, C. T., Parrillo, J. E., Guzzi, L., Henderson, S. J., Pothirat, C., Mehta, P., Fareed, J., Talwar, D., Tsuruta, K., Gorelick, K. J., Osawa, Y. & Kaul, I. (2013). A randomized, double-blind, placebo-controlled, Phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. *Crit Care Med* **41**, 2069-79.
- [196] Eguchi, Y., Gando, S., Ishikura, H., Saitoh, D., Mimuro, J., Takahashi, H., Kitajima, I., Tsuji, H., Matsushita, T., Tsujita, R., Nagao, O. & Sakata, Y. (2014). Post-marketing surveillance data of thrombomodulin alfa: sub-analysis in patients with sepsis-induced disseminated intravascular coagulation. *J Intensive Care* **2**, 30.
- [197] Shirahata, A., Mimuro, J., Takahashi, H., Kitajima, I., Tsuji, H., Eguchi, Y., Matsushita, T., Kajiki, M., Honda, G. & Sakata, Y. (2014). Recombinant soluble human thrombomodulin (thrombomodulin alfa) in the treatment of neonatal disseminated intravascular coagulation. *Eur J Pediatr* **173**, 303-11.
- [198] Yamakawa, K., Aihara, M., Ogura, H., Yuhara, H., Hamasaki, T. & Shimazu, T. (2015). Recombinant human soluble thrombomodulin in severe sepsis: a systematic review and meta-analysis. *J Thromb Haemost* **13**, 508-19.
- [199] Yoshimura, J., Yamakawa, K., Ogura, H., Umemura, Y., Takahashi, H., Morikawa, M., Inoue, Y., Fujimi, S., Tanaka, H., Hamasaki, T. & Shimazu, T. (2015). Benefit profile of recombinant human soluble thrombomodulin in sepsis-induced disseminated intravascular coagulation: a multicenter propensity score analysis. *Crit Care* **19**, 78.
- [200] Levi, M. (2015). Recombinant soluble thrombomodulin: coagulation takes another chance to reduce sepsis mortality. *J Thromb Haemost* **13**, 505-7.
- [201] Hayakawa, M., Yamakawa, K., Saito, S., Uchino, S., Kudo, D., Iizuka, Y., Sanui, M., Takimoto, K., Mayumi, T., Ono, K. & Japan Septic Disseminated Intravascular Coagulation study, g. (2016). Recombinant human soluble thrombomodulin and mortality in sepsis-induced disseminated intravascular coagulation. A multicentre retrospective study. *Thromb Haemost* **115**.
- [202] Dellinger, R. P., Levy, M. M., Rhodes, A., Annane, D., Gerlach, H., Opal, S. M., Sevransky, J. E., Sprung, C. L., Douglas, I. S., Jaeschke, R., Osborn, T. M., Nunnally, M. E., Townsend, S. R., Reinhart, K., Kleinpell, R. M., Angus, D. C., Deutschman, C. S., Machado, F. R., Rubenfeld, G. D., Webb, S., Beale, R. J., Vincent, J. L., Moreno, R. & Surviving Sepsis Campaign Guidelines Committee including The Pediatric, S. (2013). Surviving Sepsis Campaign: international

- guidelines for management of severe sepsis and septic shock 2012,. *Intensive Care Med* **39**, 165-228.
- [203] Oda, S., Aibiki, M., Ikeda, T., Imaizumi, H., Endo, S., Ochiai, R., Kotani, J., Shime, N., Nishida, O., Noguchi, T., Matsuda, N., Hirasawa, H. & Sepsis Registry Committee of The Japanese Society of Intensive Care, M. (2014). The Japanese guidelines for the management of sepsis. *J Intensive Care* **2**, 55.
- [204] Wada, H., Asakura, H., Okamoto, K., Iba, T., Uchiyama, T., Kawasugi, K., Koga, S., Mayumi, T., Koike, K., Gando, S., Kushimoto, S., Seki, Y., Madoiwa, S., Maruyama, I., Yoshioka, A. & Japanese Society of Thrombosis Hemostasis, D. I. C. s. (2010). Expert consensus for the treatment of disseminated intravascular coagulation in Japan. *Thromb Res* **125**, 6-11.
- [205] Wada, H., Japanese Society of Thrombosis Hemostasis, D. I. C. s., Okamoto, K., Iba, T., Kushimoto, S., Kawasugi, K., Gando, S., Madoiwa, S., Uchiyama, T., Mayumi, T. & Seki, Y. (2014). Addition of recommendations for the use of recombinant human thrombomodulin to the "Expert consensus for the treatment of disseminated intravascular coagulation in Japan". *Thromb Res* **134**, 924-5.
- [206] Krishnan, R., Tsubery, H., Proschitsky, M. Y., Asp, E., Lulu, M., Gilead, S., Gartner, M., Waltho, J. P., Davis, P. J., Hounslow, A. M., Kirschner, D. A., Inouye, H., Myszka, D. G., Wright, J., Solomon, B. & Fisher, R. A. (2014). A bacteriophage capsid protein provides a general amyloid interaction motif (GAIM) that binds and remodels misfolded protein assemblies. *J Mol Biol* **426**, 2500-19.
- [207] Barber, M., Tait, R. C., Scott, J., Rumley, A., Lowe, G. D. & Stott, D. J. (2004). Dementia in subjects with atrial fibrillation: hemostatic function and the role of anticoagulation. *J Thromb Haemost* **2**, 1873-8.
- [208] Murthy, S. B., Jawaid, A., Qureshi, S. U., Schulz, P. E. & Schulz, P. E. (2009). The apolipoprotein 2 allele in Alzheimer's disease: suggestions for a judicious use of antiplatelet and anticoagulant medications. *J Am Geriatr Soc* **57**, 1124-5.
- [209] Ahn, H. J., Glickman, J. F., Poon, K. L., Zamolodchikov, D., Jno-Charles, O. C., Norris, E. H. & Strickland, S. (2014). A novel Abeta-fibrinogen interaction inhibitor rescues altered thrombosis and cognitive decline in Alzheimer's disease mice. *J Exp Med* **211**, 1049-62.
- [210] Eisenberg, D. & Jucker, M. (2012). The amyloid state of proteins in human diseases. *Cell* **148**, 1188-203.
- [211] Bembde, A. S. (2012). A study of plasma fibrinogen level in type-2 diabetes mellitus and its relation to glycemic control. *Indian J Hematol Blood Transfus* **28**, 105-8.