Consequences of trauma on circulating cells and the plasma cascade systems

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Within the first hour after major injuries responses associated with the circulating cells already take place. More than 20 years ago Schlag and co-workers performing lung biopsies in trauma victims, found a pronounced accumulation of leukocytes in the lungs [1]. This leukostasis was associated with aggregation of white cells, degranulation and release of the intracellular components. Such components include endogenous inflammatory mediators, oxygen free radicals and granulocyte enzymes such as elastase which are responsible for the activation of several plasma protease systems [2,3,4,5,6,7,8,9].

The proteolytic involvement in trauma causes changes in the hemostatic balance following activation of the coagulation and the fibrinolytic system [2,3,5,7]. Furthermore, activation of the plasma kallikrein-kinin system results in the generation of vasoactive substances responsible for hemodynamic and permeability changes [8,9,10].

Accumulating data also emphasise the central role of complement activation in trauma and the importance of this cascade system during the very early stages after the injury [6,11]. Activation of this protease system has also been shown to be dependent on the extent of the injury [7,8].

The most likely activators of complement in trauma victims are injured and ischemic tissue together with unspecific proteolysis. In cases with destruction of the gut barrier, bacterial products such as endotoxin/lipopolysaccharide (LPS) might initiate complement activation and activation of circulating cells together with activation of other plasma protease systems following injury.

Several studies have also indicated that trauma produces significant damage to the endothelium. An endothelium derived vasoactive peptide using a highly sensitive and specific radioimmunoassay for human endothelin, Hirata and co-workers found a marked increase in circulating endothelin, after surgical trauma [12].

Activation of circulating cells and the plasma cascade systems have also been associated with the development of complications following trauma such as the adult respiratory distress syndrome (ARDS) and the development of multiple organ failure (MOF) [4,7,8,9,11].

Cellular activation

Activation of granulocytes in trauma victims is most likely induced by the complement anaphylatoxins C3a and C5a [6,13,14]. These products of complement activation and the terminal complement complex have also been found to be activators of monocytes and macrophages. These cells are responsible for release of cytokines including interleukins and tumor necrosis factor in trauma victims.

Macrophages play a central role in the body response to injury. Several investigators have discovered increased concentrations of the proinflammatory cytokine tumour necrosis factor α (TNFα), interleukin 1 (IL-1), and interleukin 6 (IL-6) after severe injuries [12,15,16]. Macrophages are probably also responsible for increased prostaglandin E_2 production, which can suppress lymphocyte activation [13,17]. It has been proposed that one of the primary inducers of this activation of macrophages may be bacterial endotoxin/lipopolysaccharide (LPS), but it is not clear whether injuries directly cause alterations in cell mediated immunity which in turn promotes endotoxin release and bacterial translocation or, alternatively, whether these conditions allow increased bacterial invasion which subsequently inhibits cell mediated immunity.

The proinflammatory cytokines activate the vascular endothelium resulting in the expression of adhesion molecules including the intercellular adhesion molecule (ICAM) and the endothelial leukocyte adhesion molecule (E-selectin) [18]. Activated granulocytes and monocytes express adhesion determinants such as the CD11/CD18 complex, which might bind to the endothelial adhesion molecules [19]. The discovery that these cellular molecules induce leucocyte endothelial adherence has provided a molecular understanding for leukostasis within various organs in trauma patients.

Accumulating data now indicate that the leukocyte-endothelial interaction results in increased vascular per-

meability and endothelial cell injuries which are probably important factors in the development of organ failure.

Severe trauma also has a major impact on immune competent cells. In the work by Fosse and co-workers on multitraumatised patients with a injury severity score (ISS) of 25, the total number of lymphocytes were found to be markedly reduced within the first 24 hours after the injury [20]. This was mainly due to a reduction in the number of circulating T-lymphocytes. In the patients with the most severe injuries (ISS > 16) a significant reduction in the ratio of T-helper/T-supressor cell, indicating impaired immunity was also found. The work by Faist and coworkers has largely extended these observations demonstrating decreased natural killer (NK) cell and lymphokine activated killer (LAK) cell function after injury [21].

Holzheimer and co-workers have shown that in burned mice production of interleukin-2 (IL-2) is suppressed and the macrophage proinflammatory cytokines IL-1, IL-6 and TNFα are hypersecreted in response to stimulation with lipopolysaccharide 4 to 10 days after a burn, but return to normal if the animals do not receive any further challenge [22].

In a recent experimental study using a mouse model, the same investigators found that after double challenge (burn followed by caecal ligation and puncture) there were significant reductions in the production of TNFα and IL-6 compared with caecal ligation and puncture alone, burn alone and controls [23]. These findings indicate that the activation of macrophages was reduced after infection: production of TNFα, IL-1 and IL-6 by splenocytes stimulated by LPS was also reduced. It has also been demonstrated that neutrophil functions resulting in impaired phagocytosis and chemotoxins are depressed in traumatized patients.

The powerful proteolytic enzyme elastase released from granulocytes appears to have an important pathophysiological role in trauma. When this enzyme is released into the plasma, complexes between α_2 protease inhibitor (α_2-P1) and the enzyme are formed. In clinical studies plasma values for this complex have been found to give information on the severity of organ failure and information of prognostic value in traumatized patients with sepsis [24]. Other powerful proteolytic enzymes such as cathepsins are released from granulocytes. Furthermore, these cells are sources for myeloperoxidase and lactoferrin which are of importance for free oxygen radical formation.

Altogether these observations show that the multitraumatised patient is subjected to an extensive downregulation of cellular mediated immunity resulting in immune incompetence.

The infectious threat

Infection and particularly sepsis are major threats to the traumatised patient. In the very early stages of multiple trauma bacteria and bacterial products can enter the blood stream due to lesions disrupting the surface barrier or because of injuries to the intestine. Furthermore, bacteria and bacterial products can enter the blood stream in these patients due to translocation. Translocation is the process wherein bacteria normally confined to the GI-tract cross the intestinal mucosa and appear in the mesenteric lymph nodes and other organs.

The translocation concept was put forward by Fine and his co-workers more than 30 years ago [25]. These scientists proposed that shock, burns, trauma, and inflammatory bowel disease can increase the permeability of the gut to microorganisms and their LPSs.

More recent experiments have revealed that the incidence of bacterial translocation to mesenteric lymph nodes is directly related to the presence of LPS intraperitoneally.

Studies by Wilmore's group in healthy humans have also shown that circulating LPS increases intestinal permeability [26]. Subjects, who received 4 ng/kg b.w. of *E. coli* LPS as an intravenous bolus injection experienced a two-fold increased systemic absorption and excretion of lactulose, which they received orally before exposure to circulating LPS. Recent clinical studies in trauma patients suggest that bacteria and LPS are capable of crossing the gut mucosal barrier and spreading into the systemic circulation. Thus, Rush and co-workers studying blood samples which were collected within three hours after admission in fifty patients treated in a trauma center found a high rate of positive bacterial blood cultures [27]. In the subgroup of patients with systemic blood pressure of 80 mmHg or less, fifty-six percent had a positive blood culture. Plasma LPS was detected in two of these patients. Recent experimental investigations in primates have also provided evidence of a relationship between microorganisms of the GI-tract and severe systemic infections [28].

The LPS, which is a complex glycolipid of the outer membrane of gram negative bacteria, is believed to be of great importance for initiating host responses leading to severe sepsis. LPS is not intrinsically toxic, but acts by inducing myeloid and/or nonmyeloid cells to express a multiplicity of genes encoding proteins with activities which produce the hemodynamic and hematologic changes observed in sepsis. These include cytokines, adhesive proteins, and enzymes which produce low molecular weight proinflammatory mediators [29]. LPS is one of the most potent biological response modifiers known, and picomolar concentrations are sufficient to stimulate cells of the immune, inflammatory, and vascular systems.

LPS is composed of two chemically diverse structural regions: the hydrophilic repeating polysaccharides of the core and O-antigen structures and a hydrophobic domain known as lipid A [29]. LPS binding protein (LBP), a serum glycoprotein, binds to LPS via the lipid A. This LPS-LBP complex subsequently binds to and activates the membrane receptor CD14. LBP appears to have two functional domains, one for LPS binding and another which foster LPS-CD14 interactions [30,31]. CD14 is a glycosylphosphatidylinositol (GPI)-anchored membrane protein (mCD14) of myeloid cells (monocytes and macrophages) but is is also found as a soluble serum protein (sCD14) lacking the carboxy-terminal GPI-an-

chor. In contrast to mCD14 activating myeloid cells sCD14 participate in the activation of nonmyeloid cell types, such as endothelial or epithelial cells that normally do not express mCD14 [29].

The functional LPS receptor of myeloid cells is thought to be multimeric. This multimeric receptor is believed to be comprised of a functional CD14 which binds the LPS-LBP complex and a presently unidentified transmembrane protein. Together the components of the multimeric receptor mediate the LPS signal and initiate cell activation probably via protein kinase cascades [29].

In a group of surgical intensive care patients, where more than fifty percent were treated for multiple trauma, we found that forty-nine percent of the patients had a positive LPS test during the ICU stay [32]. Using a LPS assay with a detection limit of 15 ng/l, LPS concentrations up to 850 ng/l were found. More than eighty percent of the patients had however, LPS values lower than 200 ng/l. In the patient with the highest concentration of LPS a partial liver resection had been performed. A relationship between severity and the time following trauma was found, as the percentage of patients with a positive LPS test was strikingly high during the first few days after inclusion among patients who died in multiple organ failure compared to patients with sepsis who survived and ICU patients without sepsis or organ failure. Relating mortality to the plasma LPS concentrations, we found that mortality was thirteen percent in patients with a negative LPS test during the whole observation period. Mortality was twenty-five percent in patients with a maximum LPS concentration of less than eighty ng/l, whereas patients with a plasma concentration above eighty ng/l had a mortality of forty-six percent. These figures are also in accordance with previous work by Ledingham and co-workers.

The importance of LPS in patients developing sepsis has also been confirmed by Parrillo and co-workers, who studied 100 patients with septic shock [33]. They detected plasma LPS in forty-three of the hundred patients, and plasma LPS frequently occurred in the absence of gram negative bacteremia. Thirty-seven of the patients had a positive blood culture during the observation period. In this study, ten patients with non-septic shock were also included. Detectable plasma LPS occurred in only one of the ten patients. All patients who survived became LPS negative. A cumulative percentage of patients revealed detectable plasma LPS with time as only twenty patients were endotoxemic at study entry and another twenty patients developed positive plasma LPS tests during the next twenty hours.

Effects on the plasma cascade systems

Within the first few hours after LPS administration to human volunteers marked changes in the coagulation and fibrinolytic pathway have been observed. The process of blood coagulation is also triggered by vessel injury followed by adherence of platelets to the exposed subendothelium [34]. Exposed tissues or macrophages present tissue factor to the blood, thereby activating the extrinsic pathway of blood coagulation. In the extrinsic system factor X (FX) is converted to the active protease FXa by active factor VII (FVIIa) and tissue factor (TF), a glycoprotein formed in endothelial cells (Figure 1).

The intrinsic pathway can also be activated simultaneously as a consequence of contact between plasma proteins and the subendothelium. Interactions of the contact system results in the conversion of factor XII (Hageman factor, FXII) to the α and β FXIIa (FXIIa and FXIIf) active serine proteases [35]. FXIIa activates coagulation factor XI to FXIa, which then converts factor IX to FIXa. In the presence of factor VIIIa, FIXa converts factor X to FXa. Following this activation of FX the intrinsic and extrinsic pathways are identical. FXa generates thrombin from prothrombin and thrombin subsequently cleaves fibrinogen to fibrin monomers capable of forming a fibrin clot by polymerization.

To avoid uncontrolled coagulation, the coagulation system is regulated by plasma protease inhibitors [34,36]. The inhibitory activity of antithrombin III (ATIII) which is one of the most important inhibitors of the blood coagulation system is dramatically increased by the presence of heparin. Tissue factor pathway inhibitor (TFPI), controls the extrinsic activation process. Protein C and its cofactor protein S inactivate FVIIIa and FVa, while C1-inhibitor (C1-inh.) and α_1-protease inhibitor (α_1-PI) are the major inhibitors of FXIIa and FXIa respectively (Figure 1).

Activation of the fibrinolytic system results in activation of plasminogen to the active protease plasmin. The main function of plasmin is the proteolytic breakdown of the coagulation product fibrin to fibrin degradation products (FDP) resulting in blood clot dissolution. The fibrinolytic enzyme plasmin is generated from the circulating zymogen plasminogen mainly by two pathways involving plasminogen activators (PAs) [37]. Of these the two serine proteases urokinase (u-PA) and tissue plasminogen activator (t-PA) have been well characterized. Both u-PA and t-PA are released as proenzymes which vary in functional activities, from inactive zymogens to fully active enzymes. t-PA is responsible for the extrinsic pathway of fibrinolysis.

The release of t-PA into the circulation from endothelial cells, is stimulated by various local and systemic stimuli such as exercise, venous occlusion, presence of thrombin, bradykinin, histamin, and hypoxia and acidosis. t-PA binds firmly to fibrin, making it highly thrombus specific.

Plasma protease inhibitors regulate and control activation of the fibrinolytic system at several levels [38]. Plasminogen activator inhibitors (PAIs) inhibit both PAs and their precursors. At least two specific PAIs, PAI-1 and PAI-2, are characterized. PAI-1 is released by platelets and probably acts as an acute phase reactant, while PAI-2 is released by monocytes and macrophages. The main inhibitors of plasmin are α_2-macroglobulin (α_2-M) and α_2-antiplasmin (α_2-AP).

Contact activation represents a common mechanism for the initiation of several body defences against injury, such as intrinsic blood coagulation, fibrinolysis, the plasma kallikrein-kinin system, and complement activa-

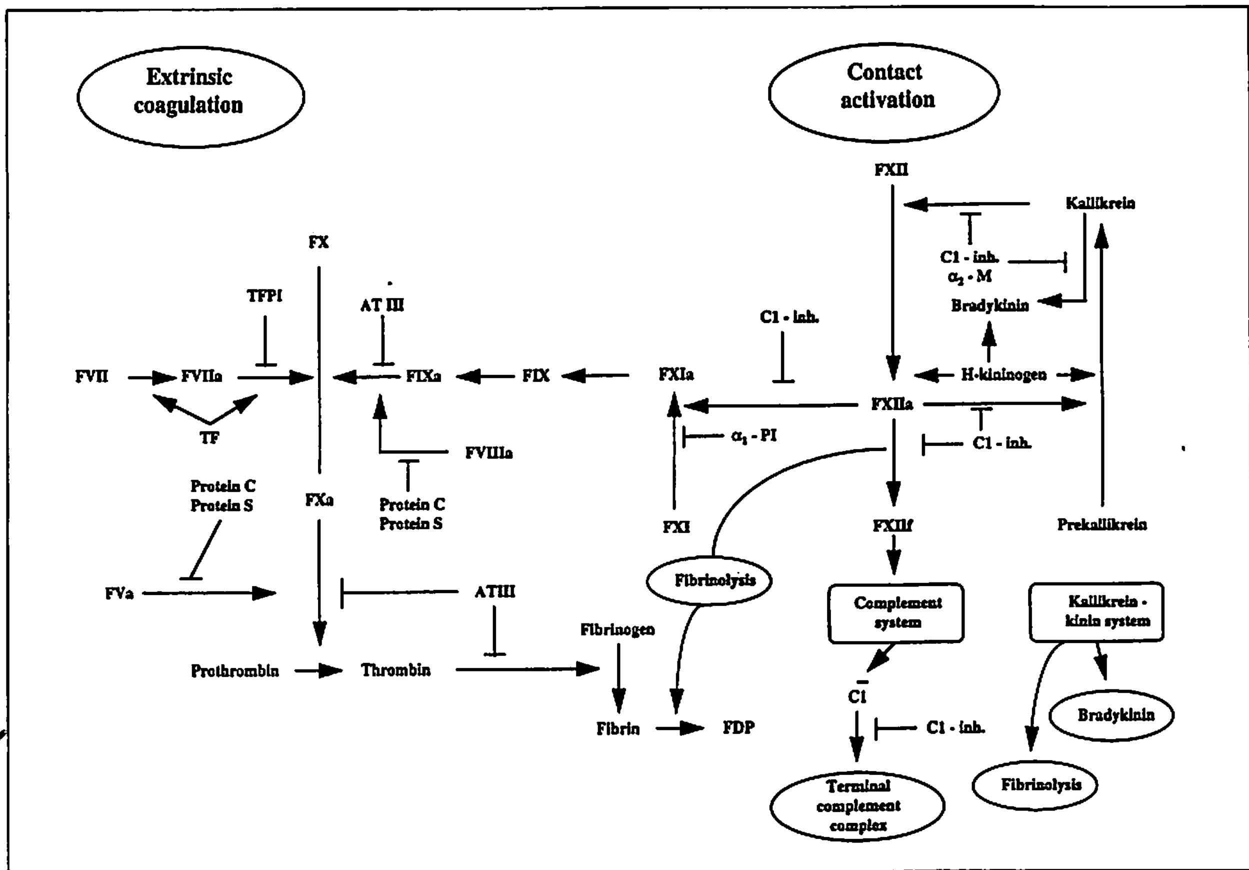

Figure 1. Interactions between the plasma protease systems.

tion. In the cascade reactions of contact activation, the zymogens FXII, FXI and prekallikrein (PKK) are converted to active serine proteases by limited proteolysis. High molecular weight kininogen (HK) is a nonenzymatic cofactor in these conversions. Binding of the FXII to a negatively charged surface (subendothelium) where autoactivation of FXII occurs, probably initiates activation of the contact system. Contact activation initiates the intrinsic coagulation cascade through activation of FXII and FXI. Plasma kallikrein can diffuse and hydrolyze its substrate HK to HKa composed of a heavy and a light chain linked by disulfide bonds. The coagulation activity resides in the light chain, which contains both the surface-binding regions and the domain responsible for complex formation between PK and FXI. FXII in plasma is converted to α and β FXIIa. βFXIIa retains the active site of FXIIa but not the surface-binding domain.

Plasma kallikrein can cleave its natural substrate HK, releasing the very potent vasodilator bradykinin (BK) [39]. Kinins are responsible for several physiological effects such as increase of vascular permeability, induction of hypotension, smooth muscle contraction, stimulation of cellular glucose uptake, provocation of pain, and t-PA release. C1-inh. and α_2-M control the release of kinins from HK while kininases control the plasma level of kinins by enzymatic inactivation. The kininogens have been shown to be potent protease inhibitors of cystein proteases such as cathepsins, papain and calpain [40]. Thus kininogens are multifunctional proteins which serve as substrates for kallikrein and release kinins, bind and assemble coagulation zymogens on activating surfaces, allowing contact activation to occur, and inhibit cysteine proteases.

Bradykinin stimulates the release of tissue plasminogen activator (t-PA) from the vascular endothelial cells [41]. This kinin action provides a link between the kallikrein-kinin system and the extrinsic pathway of fibrinolysis. Kallikrein and αFXIIa are believed to contribute to the generation of urokinase plasminogen activator (u-PA) from its inactive precursor pro-u-PA thereby interlinking the contact and kallikrein-kinin systems with the intrinsic pathway of fibrinolysis.

The complement system is activated and controlled as a consequence of contact activation [35,42]. βFXIIa activates the first component (C1) in the classical pathway of complement, in a manner analogous to the activation by antigen-antibody complexes. Plasmin has also been shown to be capable of activating C1 [43], while plasma kallikrein has been shown to inactivate it [44]. C1-inh., the major inhibitor of activated C1, also inhibits the activities of α- and βFXIIa, kallikrein and plasmin. A wide variety of mediator substances having pronounced effects on the vasculature are formed during complement activation .

Both experimental and clinical studies have shown that following trauma, the plasma cascade systems become activated [11]. The release of thromboplastin followed by activation of the extrinsic pathway of coagulation has been demonstrated in several experimental models. This leads to clot formation in various organs. The formation of microemboli in the lung following trauma was recognized in experimental studies many years ago

[3]. Thus, coagulation changes were associated with the development of pulmonary insufficiency following trauma. In experimental studies infusing thrombin intravenously leads to extensive lung injuries characterized by accumulation of micro-emboli, platelets, and leukocytes. These changes are also accompanied by increases in pulmonary vascular permeability. In the experiments performed by Malik and co-workers, decreases in leukocyte counts following intravenous infusion of thrombin was found to be highly dependent on the plasma content of fibrinogen [45]. Fibrin deposition as an element of microemboli formation has also been found to be a prominent feature in humans developing pulmonary insufficiency after trauma. In the studies of Schlag and co-workers on posttraumatic respiratory insufficiency, lung biopsies taken from patients from three hours to nineteen days after the injury demonstrated interalveolar fibrin extravasation as a part of the morphological changes.

In studies in patients undergoing total hip replacement, increased thromboplastin expression following the surgical trauma have also been reported [46]. In thirty elective cases, who underwent intra-abdominal operations, serial determinations of fibrinopeptide A and fibrinopeptide B beta 15-42 revealed significant increases during surgery. During the same time period, elevated plasma values for thrombin-antithrombin III complexes were found. The changes in these molecular markers of coagulation were, however, transient of low magnitude and followed by fibrinolysis.

In a group of multitraumatized patients, we noticed significant reductions in FXII within the first day after trauma [47]. During the same time period AT III values were also reduced. In the survivors, these parameters were normalized within the first five days after injury. In non-survivors, however, both FXII and AT III values remained reduced or declined during the observation period. The group of non-survivors were also characterized by a high frequency of detectable soluble fibrin in serum.

Activation of fibrinolysis has been recognized in different experimental models following trauma. In the work by Saldeen and co-workers [48] and by Risberg and his group [5], changes in fibrinolytic activity within the lung have been noticed after the injury. In experimental models on pulmonary insufficiency fibrin deposition has been located close to the vascular endothelium in pulmonary capillaries. The breakdown of fibrin resulting in fibrin degradation products has been associated with increased capillary permeability and formation of oedema in the lung [49]. Experimental studies have also demonstrated changes in inhibitor activities of fibrinolysis, which might be of importance for formation of microemboli in various organs.

Following a surgical trauma changes in inhibitor activities of fibrinolysis have also been observed. On the first day after major abdominal surgery, Kluft and co-workers noticed marked increases in values of PAIs. During the same time period, t-PA activity decreased [50]. Within the next two days, both parameters were normalized in uncomplicated cases. After severe trauma, markedly elevated values of PAI were found on the first day after the injury. Antiplasmin values have also been found to show an increasing pattern during the first few days in patients following severe trauma [47]. These observations have been further extended in studies on patients undergoing total hip replacement [51]. In these patients, the decreases in t-PA activity together with raised values of PAI were also found immediately postoperatively. These investigators observed that patients developing deep vein thrombosis postoperatively had higher levels of PAI postoperatively, than patients not developing this complication. Thus, changes in activity of this inhibitor appear to be of importance for the development of this serious complication after the surgical trauma. In addition, Kambayashi and co-workers observed significant increases of plasmin α_2 antiplasmin complexes during surgery indicating that activation of fibrinolysis had occured [52].

Significant reductions in plasma prekallikrein values have been found immediately after severe trauma [47]. In these patients, gel filtration studies of plasmas demonstrated plasma kallikrein-α_2M complex formation. These observations showing activation of this cascade system were further strengthened by the finding that FXII values were significantly reduced in these patients immediately after trauma. Functional plasma kallikrein inhibition values were, however, found to be within the normal range during the first week after the injury.

In experimental studies combining hemorrhagic hypotension and clamping of the portal triad, a gradual decrease of PKK values has been seen together with significantly elevated plasma kallikrein activities [7,8]. The morphological changes in the lung were characterised by perivascular and intraseptal oedema, scattered microthrombi, and trapping of leukocytes in lung microvessels. The animals gradually developed circulatory collapse. During this stage of the experiments, significant reduction of functional plasma kallikrein inhibition together with significantly reduced HK values were also seen.

LPS administration to normal humans has also been found to induce activation of the plasma protease systems. In the study by Soffredini and co-workers, LPS administration was accompanied by a seven-fold increase in plasmin α_2 antiplasmin [53]. Three hours after the injection of LPS, a six-fold rise in PAI-1 was seen together with a marked decline in t-PA activities.

The investigations by van Deventer and co-workers also revealed that marked increases in plasmin-α_2 antiplasmin complexes coincided with elevated t-PA values, indicating activation of the fibrinolytic pathway in human volunteers receiving LPS intravenously [54]. They also observed that the fibrinolytic activity of t-PA was subsequently offset by a release of PAI. Two hours after LPS exposure, increases in prothrombin fragment levels together with thrombin-AT III complexes were found, indicating activation of the coagulation pathway. In these studies, activation of the contact system estimated by measurements of FXIIa-C1-inh. complexes and kallikrein-C1-inh. complexes, however, showed no changes from baseline values. There also appeared to be

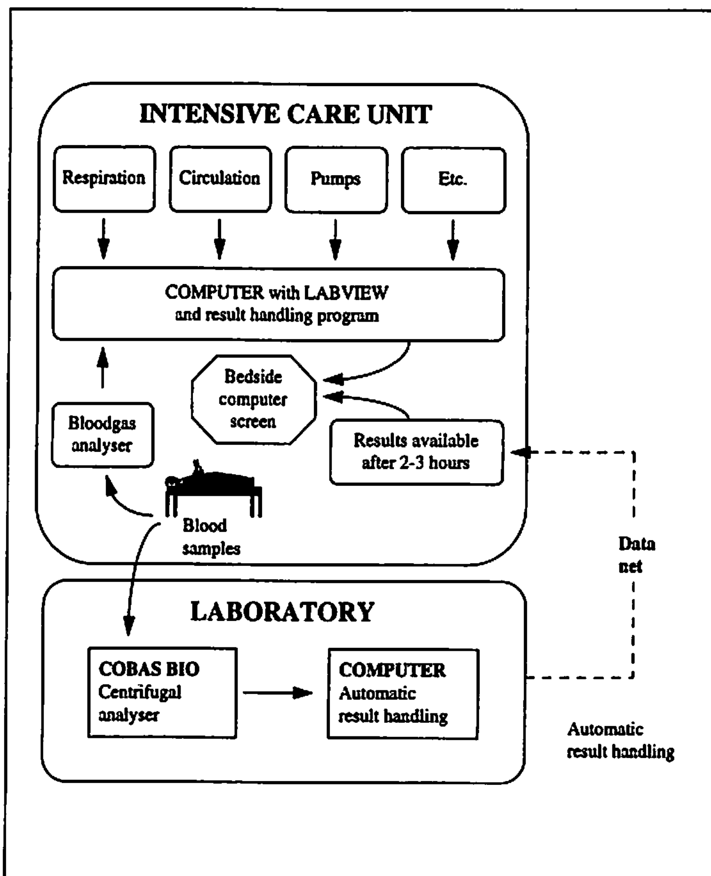

Figure 2. The Cobas – LabVIEW® monitoring system for the intensive care unit.

no activation of the complement system when evaluated by C3 activation products.

The activities of plasma proteases can be important indicators for evaluating treatment and to determine prognoses of seriously ill patients treated in intensive care units. The Cobas™ analysers are widely used for these types of analyses, and chromogenic peptide substrates are available which make the determination of a variety of proteases a simple task [10,32,47,50,55].

We have developed an automated system for result handling of the data from the Cobas™Bio analyser by connecting a computer to it (Figure 2) [56]. Two computer programs have been developed, one suited for research use and one for routine analyses of various proteases. Several advantages have been achieved.

- *Increased accuracy and security.* The results are more reliable because manual rewriting of the results is avoided. The manual transfer of results from strip chart paper to various result forms has always been an error prone procedure.
- *Standardized result presentation* is easy to obtain. Suitable graphic presentations or tables can easily be constructed, and thus the interpretation of the results facilitated.
- *"Real time" evaluation* of the results enhances the immediate impact of the results.

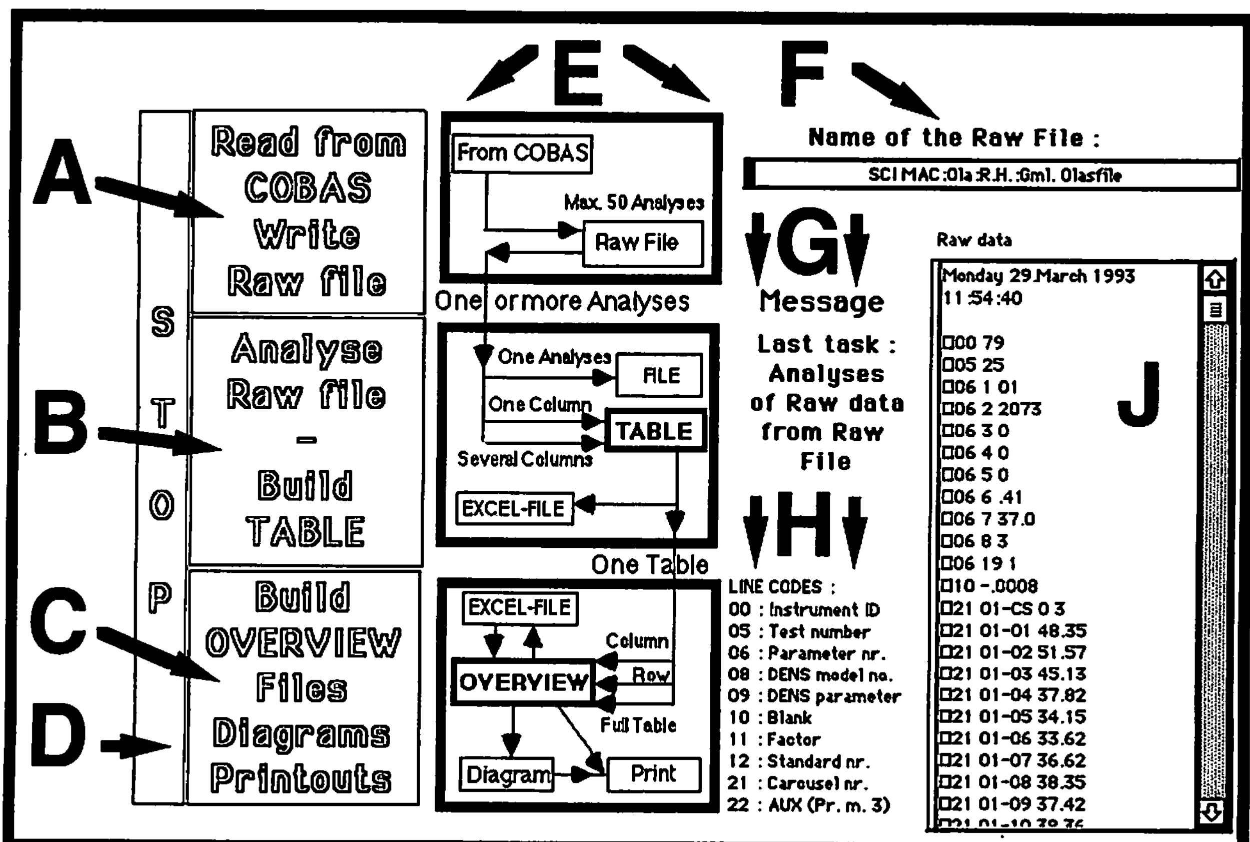

Figure 3. The main program control panel of the research oriented program. To understand the program properly, the flow chart in field E should be studied carefully. Further explanation is found in the text.

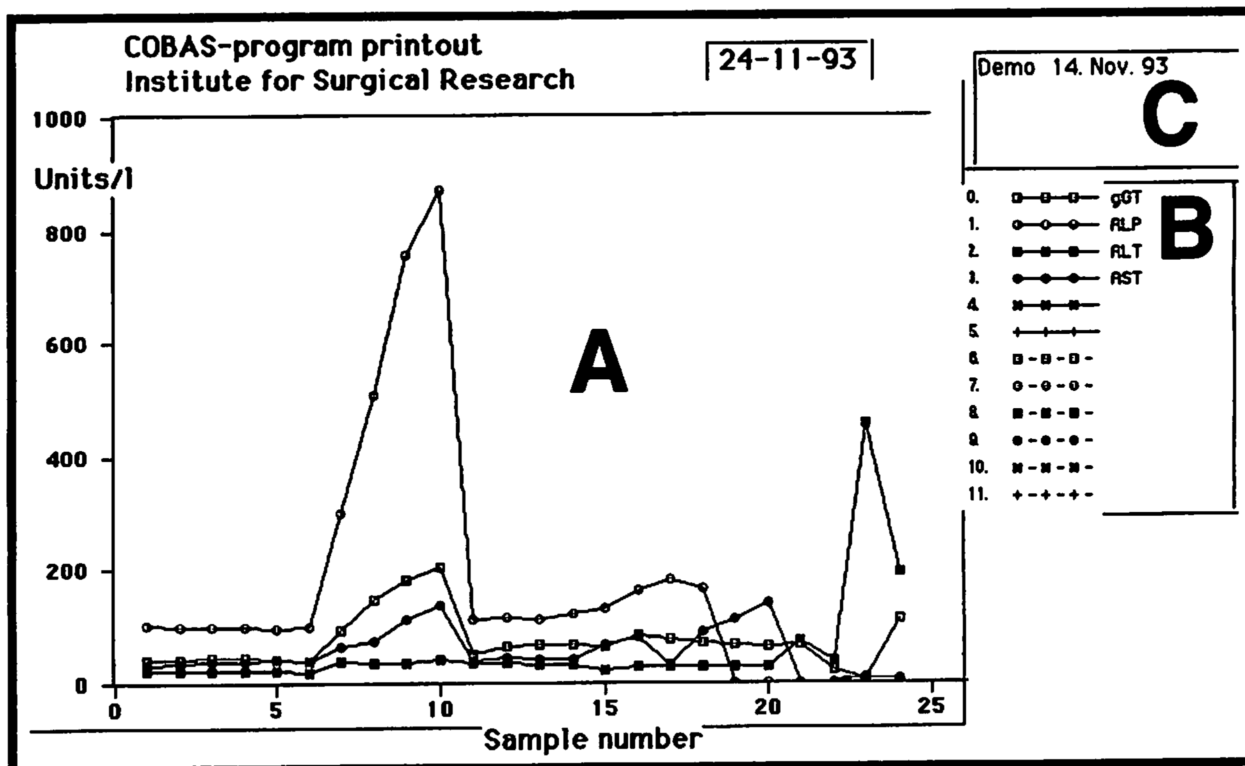

Figure 4. A graph made by the research oriented program.

Without automatic processing of results, it is very easy to postpone manual analyses. Immediate presentation of processed results increases the possibilities of obtaining early conclusions.

- *Electronic archiving of results* is a feature which can follow the introduction of computers in any experimental set-up. A machine readable archive is far more versatile than piles of paper, especially when summaries or statistics have to be made.

The user has detailed control when using the program developed for *research oriented use*. The main menu screen is shown in Figure 3 and appears when the program is started.

By means of the computer "mouse", the user points and clicks on one of the "buttons" A, B or C in the front panel picture. This action is equivalent to pressing a button on a real instrument, and directs the program flow to one of the three sub-programs, handling data *aquisition*, data *selection* and data *presentation*, respectively. These sub-programs have similar buttons to start different tasks. One button in each sub-menu will give control back to the main menu. The program can be stopped altogether by clicking the D-button. The part of the control panel VI screen marked E is a flow which that illustrates the functions of the different program parts.

The sub-menu selected with the A button will read raw data from the Cobas™ analyser, and place them in a file with the name corresponding to the contents of the field marked F. Maximally 50 analyses will be stored here, on a first-in/first-out basis (a new entry pushes out the oldest entry). The number 50 was chosen because it is well above the number of analyses reasonably performed in one day, and therefore a natural limit. However, the users can start using a new file whenever it suits them by changing the Raw File name. The users will typically change to a new file when they either needs more space because the 50-limit is approaching, or when they starts on a new series of analyses related to each other, but unrelated to previous analyses.

When results are aquired from the Cobas™ analyser, one communication session transfers one result regarding concentration of a single substance from up to 24 test tubes. This consists of between 500 and 600 bytes. Another way of using the CobasTM Bio analyser involves transferring several results for each of the 24 test tubes. This is done to determine the timing of enzymatic reactions. Depending on the number of results aquired by the analyzer during each session, the amount of data may amount to 10 kilobytes per communication session. The raw data are labeled with date and time before storage. When returning to the main menu, the raw data are displayed in the J-field. Field H is included to help the user compare "line codes" in the raw data with more meaningful information printed on the familiar strip chart recorder output. Field G indicates the latest action performed by any sub-menu after it has returned to the main menu.

Figure 4 shows a graph made by the research oriented program. The upper right corner contains a text typed by the user to identify the graph (field C). Field B indicates the type of drawing symbol used to indicate different parameters graphed in field A.

The program made for routine use automates the result handling of analyses often performed on surgically intensive care patients. Much clinical evidence has accumulated to indicate that analyses of various proteases (proenzymes, enzyme activators, enzyme cofactors and inhibitors) can provide a good indicator and prognostic tool for severely ill patients [14,32,47,57,58,59,60]. A

calculated index, the PFI index (Proenzyme Functional Inhibition index) gives reliable information on the severity of the illness [55]. The PFI index is calculated as the sum of the values (in percentage of normal values) of the six substances prothrombin, antithrombin III, plasminogen, antiplasmin, prekallikrein and kallikrein-inhibitor. Normal values are found using the six standard curves. Standard curves are obtained from pooled plasma from normal blood donors. Six hundred is subtracted from the obtained sum, giving a PFI index of around 0 in normal individuals. If one or several of the substances have a value of zero, the PFI index is set to -600 to signal an error. The program collects set of results for these six substances, as well as results for the related substances plasmin and kallikrein. Due to analytic circumstances, very high values for kallikrein or plasmin should cause a correction to be made to the raw results of prekallikrein or plasminogen, respectively. The PFI indices are calculated. The generated table of results will be printed out, and the program requests patient identification for the received results. Identifiers not earlier recognized lead to storage files for the new patient, while patient identifiers known in advance will have today's results appended to earlier results, and need no new storage files. The newly aquired results for each patient will be added to the appropriate patient file.

The routine oriented program makes or updates a file for each patient having samples analysed. It also makes a Macro-file which is used to control the Excel® spreadsheet program after the LabVIEW® program is finished. Excel® then makes a standardized result presentation on one sheet of paper for each patient. Figure 5 shows one such result summary of a patient which had 20 samples analysed. It does not matter whether the 20 samples have been analysed simultaneously or on different days, the program system will generate equal printouts as long as the patient ID is given correctly.

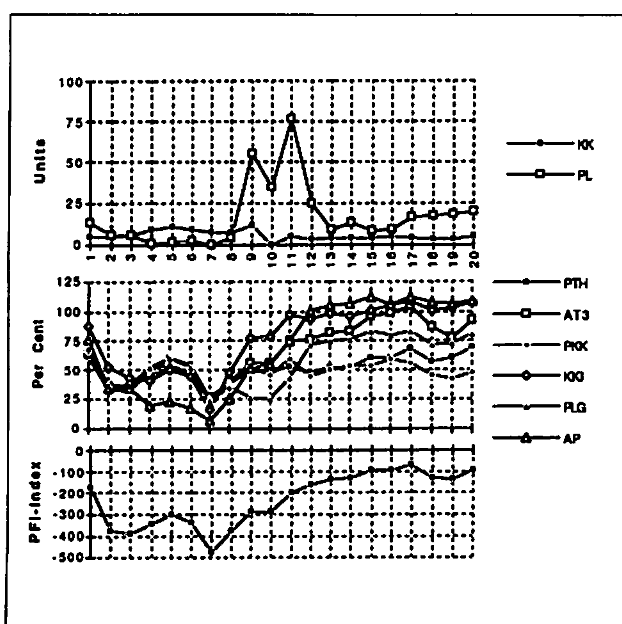

Figure 5. The Cobas – LabVIEW® monitoring system. Graphs of today's patient analyses. Further explanation is found in the text.

Development of multiple organ dysfunction and failure

Multiple organ failure is a major threat to the traumatized patient, and associated with a high mortality. As development of multiple organ failure is so strongly linked to sepsis, LPS has been subjected to significant study as a precipitating factor in the development of this condition.

In our study of sixty-one surgical intensive care patients, of whom thirty-five were treated due to severe injuries, seventy percent of the patients developing MOF according to multiple criteria had a positive LPS test [32]. In the group of patients who developed sepsis but not MOF, fifty percent of the patients had a positive LPS test, whereas only twenty-four percent of the intensive care patients without sepsis or MOF had a positive test for plasma LPS. This association between circulating LPS and the development of multiple organ failure was further strengthened when the development of MOF was studied in relation to plasma LPS concentrations. In the patients with LPS values above eighty ng/l, sixty-nine percent developed MOF. In those with LPS concentrations between fifteen and eighty ng/l, forty-four percent developed organ failure, whereas only twenty-five percent of the patients with a negative LPS test during the whole observation period had this complication.

In the group of patients with sepsis, we calculated the operating characteristic of a positive LPS test observed during the first week after inclusion in the study in predicting development of multiple organ failure. We found that the test had a sensitivity of eighty-eight percent, specificity of seventy-five percent, a positive predictive value of eighty-four percent, a negative predictive value of eighty percent, and a likelihood ratio of positive test of 3.5 in predicting multiple organ failure. This means that the chances for developing multiple organ failure in our group of sepsis patients were 3.5 times more likely in patients with a positive LPS test than in patients with a negative test for plasma LPS observed during the first week after inclusion.

Also in the studies by Danner and his co-workers, multivariant analyses revealed that renal insufficiency was 3.6 times more likely and MOF 10.3 times more likely to develop in patients with a positive test for plasma LPS compared to patients with septic shock and a negative plasma LPS test [33].

Recently, the role of plasma LPS in relation to graft survival has also been studied in humans undergoing orthotopic liver transplantation [61]. In this study on eighty-one adult patients, Yokoyama and co-workers observed that the presence of high plasma LPS values preoperatively and at the end of the anaphylactic phase was associated with graft failure and increased mortality. Also in this state, plasma LPS was considered to cause the organ failure, rather than to be an effect of this complication following surgery.

Disturbances of the hemostatic balance have been strongly associated with development of organ failure after trauma. This has been particularly studied in relation to the development of the adult respiratory distress syndrome (ARDS) [1,3,5,7,9]. The importance of a high

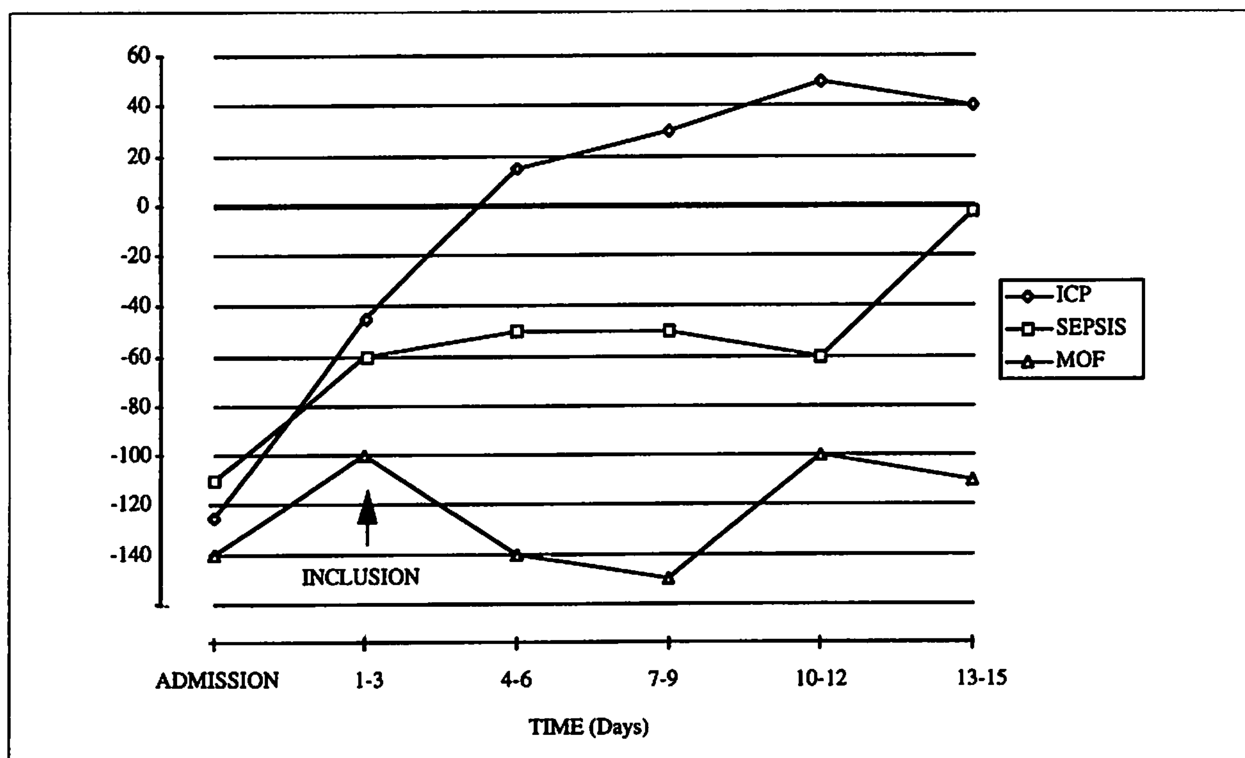

Figure 6. Proenzyme functional inhibition (PFI) index values in 61 surgical intensive care patients. ICP, Intensive care patients (without sepsis or multiple organ failure); SEPSIS, patients with sepsis; MOF, multiple organ failure.

concentration of fibrinolytic inhibitors in the blood leading to increased fibrin formation with resulting microemboli has been emphasized by several groups in the pathophysiology of pulmonary insufficiency. The importance of these cascade systems in pulmonary failure is also strongly underlined by experimental studies on permeability effects of fibrinopeptides, fibrin monomers, and fibrin(ogen) degradation products [49,62].

Studies on other components of the plasma cascade systems have also shown that the extent of protease activation might be of importance for the development of organ failure and the outcome of the disease. In a study on fifteen multitraumatized patients, significantly more reduced values for PKK, FXII, and AT III were seen within the first day after trauma in non-survivors compared to those who survived the injury [47]. In the fatal cases, a higher frequency of positive ethanol gelation test, elevated serum fibrin(ogen) degradation products (FDP), and persisting low platelet counts were also found. In more recent studies, these observations have been confirmed and extended. In a group of thirtyfive multitraumatized patients treated in the intensive care unit, plasma antiplasmin values were significantly higher within the first few days after the injury in patients developing sepsis and organ failure compared to the group of patients without such complications. Antiplasmin values remained elevated for nearly two weeks after the injury in the patients with organ failure. Determination of prothrombin and AT III using functional techniques also disclosed significantly lower values for these two parameters in the patients developing organ failure and sepsis compared to traumatised patients without these complications.

In order to improve the clinical significance of evaluating the cascade systems in patients with sepsis, the proenzyme functional inhibition index (PFI index) was designed [55]. This parameter was found to be significantly lower in patients with sepsis developing MOF than in sepsis patients without organ failure or intensive care patients without either of these complications. In the patients without organ failure, PFI index values rose rapidly towards the normal range, whereas the patients with MOF had unchanged values during the first 2 weeks of the study (Figure 6).

By calculating the operating characteristic of the PFI index in predicting MOF in sepsis patients, an index value lower than -100 observed during the first 3 days after inclusion in the study, had a sensitivity of 83%, a specifity of 68%, a positive predictive value of 91%, a negative predictive value of 75%, a likelihood ratio of a positive test of 5.8, and a likelihood ratio of a negative test of 0.19 in predicting MOF. This shows that the oods for developing MOF were 5.8 times higher in the sepsis patients with a PFI index below -100, compared to the patients having a PFI index less negative than -100.

Studies on cellular proteases from the granulocytes have also strengthened the significance of protease activation in organ failure. In the studies of Fritz and coworkers, increased concentrations of elastase-alpha-1 protease inhibitor complexes were maintained in the patients developing organ failure after surgical procedures [24]. The importance of cellular proteases for the development of organ failure has also been underlined in clinical studies by Redl, Schlag, and Goris [63, 64]. In these investigation also protease activation was associated with the development of pulmonary insufficiency.

References

1. Schlag G, Blümel G, Regele H. The use of the percutaneous needle biopsy of the lung in experimental animals and severely injured patients. In: Haberland GL, Lewis DH (eds) New aspects of trasylol therapy. Schattauer, Stuttgart. 1973;247–52.
2. Hirsch EF, Fletcher IR, Moquin R. Coagulation changes after combat trauma and sepsis. Surg Gynecol Obstet. 1971;133:393–8.
3. Saldeen T. Quantitive determination of intravascular coagulation in the lungs of experimental animals. Scand J Haematol. 1969;6:205–15.
4. Busch C, Lindquist O, Saldeen T. Respiratory insufficiency in the dog induced by pulmonary microembolism and inhibition of fibrinolysis. Effect of defibrinogenation, leucopenia, and thrombocytopenia. Acta Chir Scand. 1974;140:255–66.
5. Risberg B, Peterson HI. Pulmonary tissue fibrinolysis after drum trauma. An experimental study in the rat. Circ Shock. 1976;3:153–9.
6. Heideman M, Kaijser B, Gelin LE. Complement activation and hematologic, hemodynamic, and respiratory reactions early after soft-tissue injury. J Trauma. 1978;18:696–700.
7. Aasen AO, Saugstad OD, Lium B, Guldvog I, Lyngaas K, Larsbraaten M, Amundsen E. Plasma antiplasmin activities in experimental lung insufficiency. Acta Chir Scand (Suppl). 1980;499:113–22.
8. Saugstad OD, Aasen AO, Guldog I, Lium B, Larsbraaten M, Amundsen E. Activation of kallikrein-kinin system during experimental lung insufficiency in dogs. Acta Chir Scand (Suppl). 1980;499;123–30.
9. Malik AB. Pulmonary microembolism. Physiol Rev. 1983;63:1114–207.
10. Aasen AO. The plasma kallikrein-kinin system in multiple trauma and post-traumatic shock. In: Saldeen T (ed). Acute pulmonary insufficiency. The role of haemostatis, fibrinolysis and related mechanisms. De Gruyter, Berlin. 1985;85–102.
11. Schlag G, Redl H. The proteinases in shock. Resuscitation. 1986;14:3–21.
12. Hirata Y, Itoh K-I, Ando K, Endo M, Marumo F. Plasma endothelin levels during surgery. N Engl J Med. 1989;321:1686.
13. Goris RJA, te Boekhorst TPA, Nuytinck JKS, Gimbrere JSF. Multiple organ failure. Generalized autodestructive inflammation. Arch Surg. 1985;120:1109–15.
14. Redl H, Schlag G. Humoral and cellular activation in blood leading to organ failure. In: Schlag G, Redl H, Siegel JH (eds). Shock, sepsis and organ failure. Springer-Verlag. 1990;1151–210.
15. Nuytinck JKS, Goris RJA, Redl H, Schlag G, van Munster PJJ. Posttraumatic complications and inflammatory mediators. Arch Surg 1986;121:886–90.
16. Saldeen T. Fibrin derived peptides as mediators of increased vascular permeability. Acta Chir Scand (Suppl). 1980;499:67–74.
17. Faist E, Kupper TS, Baker CC, Chendry IH, Dwyer J, Baie AE. Depression of cellular immunity after major injury: Its association with post traumatic complications and its reversal with immuno modulation. Arch Surg 1986;12: 1000–5.
18. Pober JS, Gimbrone MA, Lapierre LA, Mendrick DL, Fiens W, Rothlein R, Springer TA. Activation of human endothelial cells by lymphokines: overlapping patterns of antigenic modulation of interleukin-1, tumor necrosis factor and ammune interferon. J Immunol 1986;137: 1893–6.
19. Smith CW, Rothlein R, Hughes BJ, Mariscalco MM, Rudloff HE, Schmalsteig FC, Anderson DC. Recognition of an endothelial determinant for CD18-dependent human neutrophil adherence and transendothelial migration. J Clin Invest. 1988;82:1746–56.
20. Fosse E. Complement activation and alterations in white blood cell populations following major surgery and trauma. 1987. Thesis. University of Oslo. ISBN 82-90522-06-1.
21. Faist E, Storck M, Ertel W, Mewes A. Posttraumatic immune suppression as initiator of organ failure. In: Schlag G, Redl H, Siegel JH, (eds). Shock, sepsis and organ failure. Springer-Verlag. 1990;307–29.
22. Holzheimer RG, Molloy RG, O'Riordain DS, Mendez VM, Curley P, Collins K, Nestor M, Saproschetz I, Mannick JA, Rodrick ML. Long-term immunotherapeutic intervention with pentoxifylline in a mouse model of thermal injury and infection. J Trauma. 1995;38:757–62.
23. Holzheimer RG, Molloy R, Mendez MV, O'Riordain D, Curley P, Nestor M, Collins K, Saproschetz I, Mannick JA, Rodrick ML. Multiple organ failure may be influenced by macrophage hypoactivitation as well as hyperactivation – importance of the double challenge. Eur J Surg. 1995;161:795–803.
24. Duswald K-H, Jochum M, Schramm W, Fritz H. Release of granulocytic elastase: an indicator of pathobiochemical alteration in septicemia after abdominal surgery. Surgery. 1985;98:892–9.
25. Fine J. Current status of the problem traumatic shock. Surg Gynecol Obstet. 1965;120:537–44.
26. O'Dwyer ST, Michie HR, Ziegler TR, Revhaug A, Smith RJ, Wilmore DW. A single of endotoxin increases intestinal permeability in healthy humans. Arch Surg. 1988;123:1459–64.
27. Rush BR Jr, Sori AJ, Murphy TF, Smith S, Flanagan JJ Jr, Machiedo GW. Endotoxemia and bacteremia during hemorrhagic shock. Ann Surg. 1987;207:549–54.
28. Schlag G, Redl H, Dinges HP, Davies J, Radmore K. Bacterial translocation in a baboon model of hypovolemic-traumatic shock. In: Schlag G, Redl H, Siegel JH, Traber DL (eds), Shock, sepsis and organ failure. Springer, Berlin Heidelberg, New York. 1991;53–90.
29. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu Rev Immunol. 1995;13:437–57.
30. Han J, Mathison J, Ulevitch R, Tobias P. LPS binding protein, truncated at Ile-197, binds LPS but does not transfer LPS to CD14. J Biol Chem. 1994;269:8172–5.
31. Weiss J, Elsbach P, Shu C, Castillo J, Grinna L, Horwitz A, Theofan G. Human bactericidal/permeability-increasing protein and a recombinant NH2-terminal fragment cause killing of serum-resistant Gram-negative bacteria in whole blood and inhibit tumor necrosis factor release induced by the bacteria. J Clin Invest. 1992;90:1122–30.
32. Aasen AO, Rishovd A-L, Stadaas JO. Role of endotoxin and proteases in multiple organ failure (MOF). Prog Clin Biol Res. 1989;308:315–22.
33. Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, Parrillo JE. Endotoxemia in human septic shock. Chest. 1991;99:169–75.

34. Colman RW, Marder VJ, Salzman EW, Hirsh J. Overview ofhemostatis. In: Colman RW, Hirsh J, Marder VJ, Salzman EW (eds). Hemostasis and thrombosis. Basic principles and clinical practice, 2nd edn. Lippincott, Philadelphia. 1987;3–17.
35. Colman RW. Surface-mediated defense reactions. The plasma contact activation system. J Clin Invest. 1984;73:1249–53.
36. Schapira M. Major inhibitors of the contact phase coagulation factors. Semin Thromb Hemost. 1987;13:69–78.
37. Bachmann F. Plasminogen activators. In: Colman RW, Hirsch J, Marder VJ, Salzmann EW (eds). Hemostatis and thrombosis. Basic principles and clinical practice. 2nd edn. Lippincott, Philadelphia 1987.
38. Kruithof EKO. Plasminogen activator inhibitors – a review. Enzyme. 1988;40:113–21.
39. Kato H, Nagasawa S, Iwanaga S. HMW and LMW kininogens. Meth Enzymol. 1981;80:172–98.
40. Müller-Esterl W, Iwanaga S, Nakanishi S. Kininogens revisited. TIBS. 1986;11:336–9.
41. Kluft C, Dooijewaard G, Emeis JJ. Role of the contact system in fibrinolysis. Semin Thromb Hemost. 1987;13:50–68.
42. Ghebrehiwet B, Silverberg M, Kaplan AP. Activation of the classical pathway of complement by Hageman factor fragment. J Exp Med. 1981;153:665–76.
43. Ratnoff OD, NAFF GB. The conversion of C1a to C1s by plasmin and trypsin. J Exp Med. 1967;125:337.
44. Cooper NR, Miles LA, Griffin JH. Effects of plasma kallikrein and plasmin on the first complement component. J Immunol. 1980;124:1517.
45. Malik AB, Fenton JW, Johnson A, Cooper J, Bizios R, Minnear FL. Thrombin induced pulmonary microembolism: mechanisms of lung vascular injury. In: Saldeen T (ed). Acute pulomary insufficiency. The role of haemostatic, fibrinolytic, and related mechanisms. De Gruyter, Berlin. 1985;33–62.
46. Høgevold HE, Lyberg T, Kierulf P, Reikerås O. Generation of procoagulant (thromboplastin) and plasminogen activator activities in peripheral blood monocytes after total hip replacement surgery. Effects of high doses of corticosteroids. Thromb Res. 1991;62:449–57.
47. Aasen AO, Kierulf P, Vaage J, Godal HC, Aune S. Determination of components of the plasma proteolytic enzyme systems gives information of prognostic value in patients with multiple trauma. Adv Exp Med Biol. 1983;156B: 1037–47.
48. Saldeen T. The microembolism syndrome. Microvasc Res. 1976;11:227–59.
49. Saldeen T. Fibrin derived peptides as mediators of increased vascular permeability. Acta Chir Scand (Suppl). 1980;499:67–74.
50. Kluft C, Verheijen JH, Jie AFH, Rijken DC, Preson FE, Sue-Ling HM, Jespersen J, Aasen AO. The postoperative fibrinolytic shutdown: a rapidly reverting acute phase pattern for the fast-acting inhibitor of tissue-type plasminogen activator after trauma. Scand J Clin Lab Invest. 1985;45:605–10.
51. Eriksson BI, Eriksson E, Gyzander E, Teger-Nilsson A-C, Risberg B. Thrombosis after hip replacement. Relationship to the fibrinolytic system. Acta Orthop Scand. 1989;60:159–63.
52. Kambayashi J, Sakon M, Yokota M, Shiba E, Kawasaki T, Mori T. Activation of coagulation and fibrinolysis during surgery, analyzed by molecular markers. Thromb Res. 1990;60:157–67.
53. Suffredini AF, Harpel PC, Parrillo JE. Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. N Engl J Med. 1989;320:1165–72.
54. Van Deventer SJH, Büller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood. 1990;76:2520–6.
55. Aasen AO. The proenzyme functional inhibition index. A new parameter for the evaluation of the severly injured and septic patient. Acta Chir Scand (Suppl). 1985;522: 211–33.
56. Sveen O, Borthne K, Aasen AO. Computer aquisition, analysis and presentation of results from a Cobas™ Bio analyser. Comput Biol Med. 1994;24:493–504.
57. Aasen AO, Smith-Erichsen N, Amundsen E. Plasma kallikrein-kinin system in septicemia. Arch Surg. 1983;118:343–6.
58. Smith-Erichsen N, Aasen AO. Evaluation of severity and prognosis in early stages of septicemia by means of chromogenic peptide substrate assays. Eur Surg Res. 1984;2:140–6.
59. Hesselvik JF, Blombäck M, Brodin B, Maller R. Coagulation, fibrinolysis, and kallikrein systems in sepsis: relation to outcome. Crit Care Med. 1989;17:724–33.
60. Witte J, Jochum M, Scherer R, Schramm W, Hochstrasser K, Fritz H. Distribution of selected plasma proteins in hyperdynamic septic shock. Intensive Care Med. 1982;8:215–22.
61. Yokoyama I, Todo S, Miyata R, Tzakis AG, Starzl TE. Endotoxemia and human liver transplantation. Transpl Proc. 1989;21:3833–41.
62. Andersson RGC, Saldeen K, Saldeen T. A fibrin(ogen) derived pentapeptide induces vasodilation, prostacyclin release and an increase in cyclic AMP. Thromb Res. 1983;30:213–18.
63. Redl H, Dinges HP, Schlag G. Quantitative estimation of leukostasis in the posttraumatic lung – canine and human autopsy data. In: Schlag G, Redl H (eds). Progress in clinical and biological research. First Vienna Shock Forum. Pathophysiological role of mediators and mediator inhibitors in shock. Liss, New York, 1987;236A:43–53.
64. Goris RJA, te Boekhorst TPA, Nuytinck JKS, Gimbrere JSF. Multiple organ failure. Generalized autodestructive inflammation. Arch Surch. 1985;120:1109–15.