CD64 (FcγRI) Expression on Neutrophil Granulocytes

A Diagnostic Marker of Acute Bacterial Infections

GUSTAV FJAERTOFT
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Abstract

Background. Newborn infants, especially preterm infants, have an increased susceptibility to serious and overwhelming bacterial as well as fungal infections. Symptoms of septicemia in especially the very preterm neonates are vague and unspecific. No really good biochemical parameter exists today that can confirm or exclude the existence of neonatal septicemia. The access to such a test in neonates would be most valuable, not only to assure early institution of effective antibiotic therapy when needed, but also to avoid unnecessary use of antibiotics, thereby reducing the risk of further development of antimicrobial resistance.

Aim. To investigate the possible use of the expression of the phagocyte receptor CD64 (FcγRI) on neutrophils for early diagnosis of bacterial infections with special reference to neonatal septicemia.

Results. Neutrophils from preterm and term newborn infants, older infants, children, and adults examined during the early phase of a bacterial infection showed a significantly higher expression of CD64 compared with non-infected controls (p<0.001). Neutrophils from even extremely preterm infants expressed CD64 to the same extent as did neutrophils from children and adult patients. The expression of CD64 was not affected by the respiratory distress syndrome (RDS) or by such factors as premature rupture of the membranes, gestational age, steroid treatment before delivery, method of delivery, birth weight or postnatal age.

Major surgery in adults (total hip replacement) did not affect the CD64 expression to an extent comparable to that found during bacterial infections. Indirectly CD64 was found to be at least equal to CRP for differentiation between Influenza A infection and bacterial infections in adults.

Conclusion. CD64 was found to be a specific and reliable marker for early detection of bacterial infections in preterm and term newborn infants, as well as after surgery. For differentiation between bacterial and viral infections it is probably at least as effective as CRP.

Keywords: CD64, FcγRI, Neutrophil, Infection, Newborn, Neonate

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


III. Fjaertoft, G., Håkansson, L., Pauksens, K., Sisask, G., Venge, P. Neutrophil CD64 (FcγRI) expression is a specific marker of bacterial infection. A study on the kinetics and impact of major surgery. (submitted)

IV. Fjaertoft, G., Pauksens, K., Håkansson, L., Xu, S., Venge, P. Cell surface expression of FcγRI (CD64) on neutrophils and monocytes in patients with Influenza A, with and without complications. Scand J Infect Dis (accepted)

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<th>Description</th>
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<tr>
<td>CL</td>
<td>chemiluminescence</td>
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<tr>
<td>CR</td>
<td>complement receptor</td>
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<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>FcγR</td>
<td>Fcγ-receptor</td>
</tr>
<tr>
<td>GA</td>
<td>gestational age</td>
</tr>
<tr>
<td>GAS</td>
<td>group A streptococci</td>
</tr>
<tr>
<td>GBS</td>
<td>group B streptococci</td>
</tr>
<tr>
<td>G-CSF</td>
<td>granulocyte colony-stimulating factor</td>
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<tr>
<td>GM-CSF</td>
<td>granulocyte-macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>HNL</td>
<td>human neutrophil lipocalin</td>
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<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin-6</td>
</tr>
<tr>
<td>IL-8</td>
<td>interleukin-8</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>ICU</td>
<td>intensive care unite</td>
</tr>
<tr>
<td>I/T ratio</td>
<td>ratio between immature and total neutrophil counts</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>interleukin-1 receptor antagonist</td>
</tr>
<tr>
<td>IVIG</td>
<td>intravenous immunoglobulin</td>
</tr>
<tr>
<td>MPO</td>
<td>myeloperoxidase</td>
</tr>
<tr>
<td>NICU</td>
<td>neonatal intensive care unite</td>
</tr>
<tr>
<td>PCT</td>
<td>procalcitonin</td>
</tr>
<tr>
<td>RDS</td>
<td>respiratory distress syndrome</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>SAA</td>
<td>serum amyloid A protein</td>
</tr>
<tr>
<td>s-ICAM-1</td>
<td>soluble intra-cellular adhesion molecule-1</td>
</tr>
<tr>
<td>TNFα</td>
<td>tumor necrosis factor α</td>
</tr>
<tr>
<td>VCAM</td>
<td>vascular cell adhesion molecule</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell counts</td>
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</table>
Introduction

During the later part of the 19th century the central role of leukocytes and phagocytosis in the defence of bacterial infection was described by scientists such as Conheim and Metchnikoff. About 50 years later the role of antibodies and serum complement began to be understood but it took even longer time until the role of lymphocytes was clarified. In contrast to this rather slow development of knowledge of the defence mechanisms against invading micro-organisms the very rapid development of cell biology during the last two decades has made it possible to elucidate on the molecular level how cells communicate with each other and with circulating factors produced by stimulated cells. One way to communicate with the environment is when a stimulated cell produces a receptor that is brought to the surface of the cell, a receptor that makes it possible for the cell to get contact with other cells or circulating factors. This knowledge, and the technical development that has made it possible to measure these receptors, is the prerequisite for my work to study the role of one such receptor on neutrophil leukocytes, and its possible role in the diagnosis and monitoring of bacterial disease, and with special reference to neonates.

Neutrophil production

Neutrophil granulocytes derive from a small pool of pluripotent haematopoietic stem cells residing mainly in the bone marrow. These cells have an extensive proliferative potential and with capacity to self-renewal and to differentiate into all haematopoietic lineages. An adult individual has to produce approximately $1 \times 10^{11}$ granulocytes per day to replace normal losses. The production, however, may be increased 10-fold or even more under conditions of stress such as acute infections. Of the total pool of neutrophil granulocytes, only 5-10% are found in the circulation.

The earliest morphologically recognisable neutropoietic cell is the myeloblast. Differentiation from myeloblast to mature granulocyte includes four to eight cell divisions and occurs normally over 7-10 days. Half of this time is spent in the post-mitotic compartment (neutrophil storage pool) as metamyelocytes, banded and segmented neutrophils. Mature neutrophils migrate from the bone marrow into the circulation through the endothelial lining of
marrow sinusoids into the blood stream, where they normally spend 6-8 hours ($t_{1/2}$) before being eliminated in the liver and spleen (1;2).

**Neutrophil function**

Neutrophil granulocytes play a key role in the first line of defence against infections, especially bacterial and fungal infections. Neutrophils, including immature forms, are released from the bone marrow in response to cytokines and other mediators of inflammation (3-5). Further steps in the neutrophil inflammatory response include adhesion, migration, phagocytosis, and microbial killing (6;7).

Adhesion of neutrophils to endothelial cells is mediated by the induction and activation of adhesion molecules on the neutrophils and the endothelial cells (8;9). Neutrophils are able to adhere to the endothelial cell adhesion molecules P-selectin, E-selectin and ICAM-1.

Adhesion to ICAM-1 is mediated through the adhesion molecules LFA-1 (CD11a/CD11b) and Mac-1 (CD11b/CD18) on neutrophils, adhesion to P-selectin by PSGL-1 (P-selectin glycoprotein ligand 1) and adhesion to
E-selectin by sialyl-Lewis^x (10;11). Adhesion to ICAM-1 is necessary for the subsequent transendothelial migration of the neutrophils.

Once in the tissue, neutrophils are attached to the site of inflammation by chemotactic factors that are generated by the micro-organisms or by the inflammatory reaction itself (12).

To enable neutrophil phagocytosis of micro-organisms they have to be opsonised by complement components and/or antibodies, which bind to specific receptors on the cell surface of the neutrophil, the complement receptors (CR1 an CR3), and the Fcγ-receptors FcγRI – III) (13;14). Following phagocytosis, micro-organisms are finally killed by the cytotoxic reactive oxygen metabolites of respiratory burst and by the action of certain granule proteins such as lactoferrin, MPO, HNL, lysozyme and BPI (15;16).

Neutrophil production and function in preterm and term newborn infants

Newborn infants, especially very preterm infants, have an increased susceptibility to serious and overwhelming bacterial infections (17;18). By its capacity for phagocytosis and killing of micro-organisms, the neutrophil granulocyte plays an important role in the defence against infections, especially bacterial and fungal infections.

Neutrophil granulocytes from newborn infants differ from adult neutrophils in many respects, quantitatively as well as functionally (19;20). Studies in newborn rats and in preterm and term newborn infants have led to the
conclusion that infants born before 32 weeks gestation have a total neutrophil cell mass that is about 20% or less compared with adult values (21-24). Both the myeloid progenitor pool and the neutrophil storage pool are reduced: in addition the regulation of neutrophil mobilisation from bone marrow is immature and not fully developed (25). Several studies indicate, however, that infants born prematurely increase their neutrophil stores per kilogram body weight to reach normal adult values already by a postnatal age of 4 weeks (26).

A number of cytokines are able to promote the proliferation and differentiation of neutrophils, among which G-CSF and GM-CSF are probably the most well known (25). These two cytokines act relatively specific on the neutrophil lineage (and for GM-CSF the monocyte lineage as well). Several studies have shown an increased serum level of both G-CSF and GM-CSF in term and preterm infants as compared with healthy adults, although the results are somewhat variable (27). On the other hand, the expression of specific receptors for G-CSF on neutrophils from newborn infants has been reported to be significantly lower compared with the expression on adult neutrophils, and reduced even more during an ongoing bacterial infection (28).

Reduced chemotaxis (cell movement towards an inflammatory stimulus) is the most frequently reported functional abnormality of neonatal neutrophils (19). Laboratory tests have shown that neonatal neutrophils display less interaction with endothelial monolayers in condition of flow than adult cells. For instance, rolling adhesion is diminished, fewer cells attach to activated endothelium, and fewer cells migrate to the subendothelial tissue (29). These abnormalities are caused by an abnormal expression and dynamics of two families of adhesion molecules, the $\beta_2$ integrins and the selectins (30,31). In addition, abnormalities in the neonatal neutrophil cytoskeleton also strongly contribute (32,33).

Several studies have shown that neutrophil migration, investigated by in vitro assays, is abnormal at birth in both term and preterm infants (34). In term infants the chemotactic function normalises quite rapidly. In preterm newborn infants, however, an increased chemotactic capacity is seen 2-3 weeks post partum: thereafter, the maturation proceeds slowly, reaching normal adult values not until 40 weeks or more post-conceptional age (35-37).

The process of phagocytosis and killing of bacteria is mediated through receptors for both complement and the Fc domain of immunoglobulins and is therefore dependent on the micro-organism being opsonised by complement or specific antibodies (38).

Most studies focusing on the capacity of neonatal neutrophils for phagocytosis of bacteria have used isolated neutrophils and bacteria opsonised with adult immunoglobulin and complement. Under such conditions, in which the target bacteria is optimally opsonised, the neutrophils from term
and preterm newborn infants have been shown to have a capacity for phagocytosis almost equal to that of adult neutrophils (39). However, when tested in an assay using whole blood, neutrophils from preterm infants had a significantly decreased capacity for phagocytosis as compared with neutrophils from both term newborn infants and adults (40).

Taken together these investigations clearly indicate that the important limiting factor for neutrophil phagocytosis in preterm infants is the low capacity for opsonisation in blood from these infants. Furthermore, the neutrophil expression of CD16 (FcγRIII), the most abundant Fcγ-receptor on neutrophils, is also significantly reduced in preterm as well as in term newborn infants (41;42;42;43).

Because nearly all trans-placental transport of IgG takes place during the last trimester, very preterm newborn infants have a serum level of IgG considerably lower than the values found in term newborns (44;45). Complement activity in very preterm infants is also markedly reduced in comparison with what is found in term neonates (46;47). Similarly the neutrophil expression of CD16 (FcγRIII) and CD32 (FcγRII), as well the capacity for up-regulation of CD11b/CD18 (CR3) is significantly lower (42;48;49). CD32 has the highest affinity of all three Fcγ-receptors for the binding of IgG2, the most important IgG subclass of antibodies against encapsulated bacteria(14). As a consequence of these findings markedly impaired neutrophil phagocytosis is to be expected in preterm infants, which has also been shown in the few studies published in recent years. However, a correspondence with the expression of Fcγ-receptors or CR3 was not found (40).

The respiratory burst represents the most important mechanism by which neutrophils kill phagocytized micro-organisms. Its activity is measured by the chemiluminescence (CL) response. Several studies have found neutrophils from term neonates to have a CL response equal to that of adult neutrophils, whereas neutrophils from preterm newborn infants have a much smaller CL response (50).

The neutrophil contains granules in which a number of different bactericidal proteins are stored and later released upon activation of the cell. The most well known of these proteins are lactoferrin and myeloperoxidase (MPO). The concentration of both these proteins is markedly reduced in neutrophils from preterm infants, probably leading to a reduced capacity for intra-cellular killing of bacteria and other potentially pathogenic micro-organisms (51).

In order to reduce the high frequency of severe infection seen in very preterm newborn infants, several attempts have been made to restore at least some of their immunological defects. Simply to increase the serum level of IgG turned out to have nearly no effect at all on neither the frequency nor the outcome of severe bacterial infections (52). However, taking into account that the organisms causing sepsis in preterm neonates are rather different from the organisms usually causing infections in adults, from whom the IV
immunoglobulin (IVIG) preparations are prepared, the lack of success using IVIG for prophylaxis of neonatal sepsis was perhaps not so surprising. Further analysis of the many studies performed on this subject has indicated a possible usefulness of IVIG for prophylaxis of neonatal sepsis in selected subgroups of patients (53). In addition, recent investigations indicate that IVIG used as additional therapy in severe neonatal sepsis might have a beneficial effect.

Like IVIG supplementation, treatment with G-CSF and GM-CSF should, theoretically, be an ideal form of prophylaxis against infection in very preterm newborns, increasing their pool of mature neutrophils as well as their neutrophil production. However, in several studies this treatment has thus far failed to show any significant effect on at least the survival from severe bacterial infections in the very preterm neonates (54) with one exception: Giving G-CSF to preterm newborns with neutropenia that was caused by maternal pre-eclampsia was found to reduce the frequency of severe infections in this selected group (55).

### Phagocyte receptors

<table>
<thead>
<tr>
<th>Complement receptors</th>
<th>Fcγ-receptors</th>
<th>Affinity</th>
<th>Cell type</th>
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<tbody>
<tr>
<td>CD11b/CD18</td>
<td>CD16 = FcγRII low neutrophils / (monocytes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD35</td>
<td>CD32 = FcγRII low neutrophils / monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD64</td>
<td>CD64 = FcγRI high (neutrophils) / monocytes</td>
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</table>

### The Fcγ-receptors

Three types of Fcγ-receptors are found on neutrophils and monocytes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) (56). Interaction between Fcγ-receptors and the Fc-portion of immunoglobulin molecules triggers various important biological functions in the cell, including phagocytosis, activation of the respiratory burst, degranulation, and antibody-dependent cell cytotoxicity (ADCC) (57-60). CD16 and CD32 are normally expressed by neutrophils and are low affinity receptors that only bind polyvalent IgG complexes. In contrast, CD64 is constitutively expressed only by monocytes and to a very low extent by neutrophils. It is a high affinity receptor, which means that it binds monomeric IgG (61).

Binding of the Fc-portion of immunoglobulin molecules to the different Fcγ-receptors stimulates different aspects of neutrophil function to a varying degree (60). In addition, polymorphism exists, making the different re-
responses even more heterogeneous (62). This polymorphism is of importance not only regarding the capacity for stimulating different parts of the neutrophil function but it has also been shown to determine to some extent the predisposition of an individual to attract certain diseases (63;64), and according to some investigations, the severeness and outcome of some infectious diseases (65;66). The various Fcγ-receptors also differ regarding such aspects as structure, attachment to the cell membrane, mechanisms by which increased receptor-expression is stimulated and possible storage in the granules of the neutrophil cell (ref). CD16, for instance, is stored in the granules and transported to the cell surface in response to certain stimuli such as infections. Lacking a stable anchoring to the cell membrane, CD16 is then shed from the cell surface as a consequence of further stimulation and therefore measurable in serum as soluble receptor (sCD16).

Several studies have found this sCD16 to be a possible marker, not only for diagnosis of infectious diseases but also for measuring the severity of the disease (67-69). Moreover, it has been shown that in patients with neutropenia the serum level of sCD16 is better correlated to the patients’ total amount of neutrophils, and also to the risk of developing severe bacterial infections, than just measuring the neutrophil blood count (68;69).

Neutrophils from preterm infants express both CD16 and CD32 to a significantly lesser extent than neutrophils from term infants and adults (42;70;71). CD16 is the most abundant Fcγ-receptor on neutrophils and CD32 is the most important Fcγ-receptor for binding of IgG2, the most significant IgG subclass in the defence against encapsulated bacteria. The reduced expression of these receptors on neutrophils from newborn preterm infants is thought to contribute to the increased susceptibility to serious bacterial infections in this age group.

**CD64 (FcγRI)**

The Fcγ receptor, CD64 (FcγRI), is a high affinity receptor normally expressed by monocytes and involved in phagocytosis and intracellular killing of bacteria (14). Neutrophils from healthy individuals express CD64 to a very low extent. However, it has been known for some time that neutrophils exposed to IFN-γ and G-CSF in vivo (72;73) or IFN-γ in vitro (74), also express CD64 . Neutrophil CD64 expression has been used for monitoring the effect of therapeutically administrated IFN-γ (75). Neutrophil stimulation by using specific anti-CD64 antibodies results in activation of the respiratory burst (61;76). The affinity of CD64 for different IgG subclasses varies as follows: IgG3 > IgG1 >> IgG4>>> IgG2. (57) During bacterial infections the CD64 expression on neutrophils was first shown to be increased in adult patients (77;78).
Neonatal septicaemia

Newborn infants, especially preterm infants, have an increased susceptibility to serious and overwhelming bacterial as well as fungal infections. This can at least partly be explained by poorly developed barrier functions, immature immune system, both humoral and cellular, and at least in the very preterm newborn infant, a far from fully developed phagocyte system (20).

Neonatal septicaemia is divided into early-onset (first 72 hours) and late-onset (72 hours – 30 days) septicaemia. Early-onset sepsis is most often caused by group B streptococci (GBS), E. coli and other Gram-negative intestinal bacteria. In late-onset sepsis coagulase-negative staphylococci (CoNS) are the most frequent causative organisms, followed by Staphylococcus aureus and Candida albicans, the latter becoming a gradually more frequent cause of neonatal septicaemia (79).

The symptoms accompanying neonatal sepsis are, at least initially, often vague and unspecific, such as: RDS-like symptoms, tachypnoea, onset- or increasing frequency of apnoea, temperature instability, feeding difficulties.

The incidence of sepsis among very preterm newborn infants varies according to different investigations (between 25 and 50 %), with low gestational weight being the most important risk factor. Other factors predisposing for development of neonatal septicaemia are prolonged rupture of the membranes, maternal fever, instrumentation and arterial or venous lines.

Diagnosis of neonatal septicaemia

A positive blood culture with known pathogenic bacteria is the gold standard for diagnosis of sepsis in the neonatal period, as well as later in life. In the neonate however, the blood culture is quite often negative although the infant is thought to suffer from septicaemia. The reason for this might be a suboptimal volume of blood for culture or antibiotics administered to the mother before delivery. Accordingly, a diagnosis of suspected sepsis has to be based on clinical symptoms together with one or two biochemical parameters such as CRP and/or a low neutrophil count. Since the symptoms of septicaemia in especially the very preterm neonates are so vague and unspecific and since no really good biochemical parameter exists today that can confirm or exclude the existence of septicaemia, antibiotic treatment has to be started in the NICUs on a very wide indication with ensuing over-consumption of antibiotics.
Laboratory tests for early detection of bacterial infections in neonates

The most commonly used biochemical tests today for early detection of neonatal septicaemia are CRP and neutrophil count. Platelet count is also used to some extent, but is unspecific since a low platelet count can be found not only in infections but also in many other conditions.

CRP is probably the single most commonly used laboratory test for early diagnosis of neonatal sepsis. It is however, well known that the CRP concentration does not rise very early during an infection and a low value does not rule out sepsis. CRP is an acute phase protein produced in the liver as a result of IL-6 stimulation. One of its advantages is that in healthy individuals its serum level is close to zero. However, the production of CR does not start until 6 - 8 hours after the onset of the infection; furthermore, it increases rather slowly in adults and even more so in preterm neonates (80;81). Serial measurements will probably increase the diagnostic power, however (82).

In addition to serving as a diagnostic test, CRP is widely used to monitor the course of the disease and the effect of treatment. Since it takes at least 2 days from when a blood culture is drawn until a causative organism is identified and its resistance pattern is known, it is of great importance to be able to detect the earliest possible signs of the effect of treatment. However, having a long half life (T1/2) of 17 hours, CRP is not an ideal parameter for this purpose, although it is commonly used to monitor the effect (83).

The blood neutrophil count is also commonly used for early diagnosis of bacterial infections among neonates, with a low value strongly indicating severe infection. Unfortunately, the neutrophil count decreases rather late and often not until the situation tends to become critical. As for CRP, the usefulness of the neutrophil count can be increased by repeated measurements, which today is perhaps the best way for surveillance of a newborn infant with a suspected up-coming infection. To improve the value of the neutrophil count for diagnosis of neonatal infections, the ratio of immature to mature neutrophils has been widely used but its low specificity has made this ratio less useful (84-86).

To compensate for the initially slow increase in CRP levels attempts have been made to combine the measurement of CRP with the measurement of IL-6. This combination seems logical in that during the initial phase of an infection the production of IL-6 proceeds and initiates that of CRP (87). The serum level of IL-6 rises very early in response to an invading organism but is a rather unspecific marker of infection; on the other hand, CRP increases more slowly but is a more specific marker. This makes the combination a fairly reliable test for early detection of severe bacterial infections in neonates. For technical reasons, IL-8 is often used instead of IL-6 with the same results (88).
Measurement of the serum levels of the cytokines IL-6 or IL-8 alone has also been studied for routine use in the diagnosis of septicaemia in newborn infants as well as later in life. These tests have a disadvantage in that the rise in serum concentration of these and other cytokines during bacterial infections is of very short duration, which makes it difficult to “catch” the peak for diagnostic purposes (89;90). Then again, a study using interleukin-1 receptor antagonist (IL-1ra) and IL-6 have shown that it is possible to detect increased levels of these cytokines as early as 48 hours before the infection is clinically recognisable (91). This knowledge will perhaps give us an opportunity to survey at least the most vulnerable newborn infants with regard to septicaemia, thereby making it possible to start treatment for an upcoming infection before it becomes a severe threat to the infant’s health.

SAA (Serum amyloid A protein) is another acute phase protein that may be used for diagnosis of bacterial infections. Like CRP, the serum level of SAA in healthy, non-infected individuals is equal to or near zero, which is an advantage when used as a diagnostic tool. In many respects the properties of SAA equal those of CRP but in some situations SAA may be superior to CRP, including being used as a diagnostic marker for neonatal sepsis (92).

PCT (Procalcitonin) is yet another serum marker used for diagnosis of bacterial infections. In general, PCT seems to have no advantage over CRP though in certain situations it has been shown to be superior. One such example is the use of PCT as a predictive marker for the outcome of severe sepsis in adult ICU patients (93). During bacterial infections, an elevated serum level of PCT can be detected fairly earlier compared with the rise in CRP concentration, making it perhaps a somewhat better early marker for bacterial infections (94). Concordantly, it has been shown that the maximum PCT level after injection of endotoxin is reached already after 6 to 8 hours (ref). On the other hand, the regress in serum concentration of PCT in response to successful antibacterial treatment is even slower than that of CRP, with an estimated T1/2 as long as 25-30 hours (95;96).

Studies in newborn infants have shown rather diverging results as to whether PCT might be a useful marker for early diagnosis of neonatal sepsis. During the first few days of life a rather small but highly variable increase in the serum level of PCT is found in healthy term and preterm infants. The levels seen during neonatal sepsis are, however, much higher (97).

Several other cytokines and acute phase proteins, (e.g., TNFα, α1-antitrypsin and lactoferrin) have also been investigated as potentially diagnostic markers for bacterial infection in the neonatal period. These markers have quite often shown a fairly good sensitivity, but most of them have been too unspecific to be superior to the other markers mentioned above (98).
Receptor molecules

The expression of several receptor molecules on neutrophils, monocytes, and lymphocytes has been investigated as potential markers for bacterial infections in both adults and newborn infants. Those receptor molecules that are shed as a consequence of the stimulation or activation of a cell and then detectable in serum as soluble receptors are of special interest. Measuring the serum concentration of these molecules is often technically much easier and less time-consuming than measuring the receptor expression on the cell surface. Soluble intracellular adhesion molecule-1 (s-ICAM-1), L-selectin, and E-selectin are all examples of soluble receptor molecules which have been evaluated as possible infection markers; and again, the lack of acceptable specificity rather than lack of sensitivity, is most often the limiting factor when it comes to practical usefulness (99;100). Among the soluble receptors S-CD16, as mentioned earlier, is probably the most usable one, being a valuable marker for diagnosis and in providing an indication of the severity of the infection (67).

Of the cell surface receptor molecules, CD11b possesses a unique property. Being stored in the intra-cellular granule, more receptor molecules are transported to the cell surface in response to a bacterial infection (101). When endotoxin binds to the endotoxin receptor CD14 on the surface of monocytes, it lasts less than 5 minutes until an increased CD11b expression on the cell surface can be measured, rendering this receptor expression a potentially valuable early marker for bacterial infections. Further investigations have shown, however, that an increase in neutrophil CD11b expression is a rather unspecific phenomenon, and not only a response to bacterial infections (102). In newborn infants an increase in neutrophil CD11b expression seems to be a rather unspecific sign of neutrophil activation seen in different situations and also related to factors such as labour length (103;104). In addition, the measurement of the CD11b expression is practically and technically somewhat complicated since each blood sample has to be placed on ice (4°C) immediately after sampling to prevent further up-regulation in vitro (105).

Recently the possible use of neutrophil and monocyte CD11b expression for surveillance of especially infectious prone, very preterm neonates has been studied, showing that an increased receptor expression could be detected up to 24 hours before any clinical sign of septicaemia was noted (105). Whereas the sensitivity of the increased neutrophil CD11b expression was high regarding an up-coming infection, the specificity was again low, being no better than 56%.
CD 64 (FcγRI) as a diagnostic marker for bacterial infections

CD64 - Fcγ-receptor I – is the only Fcγ-receptor capable of binding monomeric IgG, a phenomenon that might be of importance under conditions characterised by low concentrations of specific immunoglobulins, i.e. during the early phase of an acute bacterial infection and in very preterm newborn infants. Stimulation through CD64 leads to increased neutrophil capacity for phagocytosis and intra-cellular killing of bacteria (61).

At the time when the work on this thesis started very little information could be found in the literature about neutrophil CD64 expression. In fact, the only information available was that administration of INF-γ in vivo resulted in the appearance of CD64 on neutrophils and that the measurement of this expression could be used to monitor the biological effect of IFN-γ treatment (72;75). In addition one study had shown that neutrophils from patients suffering from tonsillitis that was caused by streptococcus group A (GAS) expressed CD64, leading to the conclusion that GAS infection is a powerful stimulation of IFN-γ production (106). Two other studies published somewhat later pointed to the possible usefulness of neutrophil CD64 expression or the increased expression of CD32 on neutrophils or CD16 on a subgroup of monocytes as diagnostic markers for bacterial infection (77;78). At the time our first article on this subject was published, showing that neutrophils from even the very preterm newborn infants during bacterial infections express CD64 to the same extent as neutrophils from adult patients, no other information about the possible value of neutrophil CD64 expression as a diagnostic tool in this age group was available. Since then, however, two larger studies have concluded that neutrophil CD64 expression is a sensitive diagnostic marker for late onset as well as early onset neonatal infection (107;108), and generally, CD64 is today considered one of the most promising diagnostic markers for bacterial infections in neonates(98).

What is more, among adult patients increasing knowledge has become available about the possible use of CD64 as a diagnostic marker. In one study neutrophil CD64 expression was found to be a useful diagnostic tool for early diagnosis of bacterial infections in patients suffering from autoimmune diseases (109). In this group of patients fever and elevated CRP are often components of the disease, making the early diagnosis of a concomitant bacterial infection difficult. Because these patients are often treated with steroids and/or other immunosuppressive drugs it is important to reach an early diagnosis of a bacterial infection.
Aims of the study

To investigate the possible usefulness of neutrophil CD64 expression for early diagnosis of bacterial infections, with special reference to infections in newborn infants.

To examine the cell surface expression of CD64 on neutrophils from preterm and term newborn infants, children and adults with and without bacterial infections.

To follow the neutrophil CD64 expression in maturing preterm neonates by repeated measurements up to the age of one month or more.

To examine the neutrophil CD64 expression in healthy term newborn infants during the first few days of life.

To study the possible influence on neutrophil CD64 expression of a major surgical trauma, i.e. total hip replacement.

To study the kinetics of neutrophil CD64 expression during the first 3 days after start of treatment of a bacterial infection in children and adults and during the first 3 days after major surgery in adults.

To investigate the ability of the neutrophil CD64 expression to distinguish between bacterial infections and infections that are caused by Influenza A virus.
Materials and methods

Patients

In paper 1 (Neutrophils from term and preterm newborn infants express CD64 during bacterial infections) three different groups of patients were studied.

One group consisted of 12 preterm and term newborn infants (GA 24-42 weeks) with culture verified (n=7) or suspected (n=5) septicaemia. Suspected septicaemia was defined as clinical symptoms in combination with negative blood culture, but with an elevated serum level of CRP and/or leucopenia. The infection started at the median age of 4 days (0 to 60 days). The first analysis of receptor expression was performed at the median of 1 day (0 to 2 days) after start of treatment and at the median of 1 day (0 to 3 days) after the onset of symptoms. From nine of these infants a second blood sample was obtained 1 - 6 weeks (median 2 weeks) after the first one.

A second group consisted of 14 infants and children, aged 10 months to 6 years, who were hospitalised because of an acute bacterial infection. Five were diagnosed as having pneumonia, 4 pyelonephritis, 2 ethmoiditis, one local abscess, one septicaemia and one suspected septicaemia. Only one child had a positive blood culture. The blood sample was obtained at the median of 1 day (0 to 5 days) after the onset of treatment and 3 days (median) after start of symptoms. From seven of the children a second blood sample was available at a median time of 3 months (6 days to 9 months) after the infection.

A third group consisted of 6 adult patients, 29 to 84 (mean 55) years of age, who were hospitalised because of an acute bacterial infection. Three had streptococcal infections (erysipelas, tonsillitis, soft-tissue abscess), and one each of pyelonephritis, Campylobacter enteritis and E. coli septicaemia. The blood sample was obtained at the median of 1 day (0 to 2 days) after the onset of treatment.

Seven non-infected preterm neonates, 14 healthy term neonates, and 26 healthy adults served as control groups.

In paper 2 (Cell surface expression of CD64 on maturing neutrophils from preterm and term newborn infants) three groups of infants and one group of adults were studied.

The first group consisted of 22 preterm newborn infants without obvious clinical signs of infection, with a median GA of 26 weeks (range 23–30 weeks) and a median birth weight of 1000 g (range 608–1555 g). The analy-
sis of the receptor expression, using capillary or arterial blood, was performed on the median of day 1 (0 to 2 days) after birth. In 11 of these infants the receptor expression was followed about once a week up to more than one month of age.

The second group included 18 healthy, term newborn infants with a median GA of 39.6 weeks (range 38–42 weeks) and a median birth weight of 3510g (range 2650–4230g). All were vaginally delivered without any known perinatal complication. The analyses were performed on cord blood.

A third group was comprised of 30 healthy adults from whom blood was drawn by venous puncture.

Group four was made up of 9 healthy term newborn infants vaginally delivered and without any known perinatal complication. In this group three consecutive analyses were performed: at birth (cord blood), by day 1 (n=9), and by days 3–5 (n=7). Capillary blood was used from day 1 and forward.

In paper 3 (Neutrophil CD64 expression is a specific marker of bacterial infection. A study on the kinetics and the impact of major surgery) three groups of patients were studied.

One group consisted of 8 children, aged 8 days to 7 years who were hospitalised because of bacterial infection. Four children suffered from pneumonia and 4 from pyelonephritis. Blood samples for analysis of the receptor expression were collected within 20 hours after admittance (the next morning) and the two following days.

A second group consisted of 19 adult patients, mean age 73 years (24 to 89 years), who also were hospitalised because of bacterial infection. Of these 7 had pneumonia, 4 pyelonephritis, 4 erysipelas, one meningitis and septicaemia, one verified and one suspected septicaemia, and one cholangitis. Blood samples for analysis of receptor expression and serum markers were collected within 20 hours after admittance (the next morning) and the two following days.

Group three consisted of 12 adult patients (8 females and 4 males), mean age 58.4 years (48 to 76 years), who were admitted to the Department of Orthopaedics, Uppsala University Hospital, for total hip-joint replacement because of coxarthrosis. Blood samples for analysis of receptor expression and serum markers were collected preoperatively and the first three days following surgery. In addition, a serum sample was collected 6 hours after the start of surgery. None of these patients had a former history of inflammatory joint disease and none had ongoing treatment with steroids or non-steroid anti-inflammatory drugs.

Group 4 was made up of 30 healthy adults.

In paper 4 (Cell surface expression of CD64 on neutrophils and monocytes in patients with influenza A, with and without complications), all patients included in the study visited the Department of Infectious Diseases at the University Hospital of Uppsala as in- or out-patients. Twenty two adult patients with influenza A were included during the winter season when there
was an ongoing influenza epidemic in the community, whereas 29 adults suffering from bacterial infections were included during the whole year. The influenza diagnosis was established clinically based on typical influenza symptoms with concomitant epidemiological data. If the diagnosis was uncertain, laboratory tests for confirmation of influenza were performed. Patients with influenza were grouped into influenza with (9 patients) and without (13 patients) complication. Those with uncomplicated influenza infection were defined as patients who were mainly observed at the hospital or sent home without any specific treatment because of their disease. Patients with influenza complications were defined as those who were treated for a complication to influenza such as suspected bacterial bronchitis or pneumonia and/or respiratory and/or cardiovascular complications.

Patients with bacterial infection consisted of 29 patients, with the following diagnoses: pneumonia (n=11), urinary tract infection (n=7), pneumonia and urinary tract infection (n=2), erysipelas (n=4), erysipelas and urinary tract infection (n=1), cholangitis (n=1), meningitis (n=2), and dental abscess (n=1). All patients with pneumonia had a positive chest x-ray and responded to beta-lactamase antibiotics. All blood samples were drawn within 24 hours after admittance.

The control group consisted of 29 healthy adults.

Methods

Preparation of leukocytes

Leukocytes were prepared according to the procedure described by Hamblin et al (110). Briefly, 0.5 or 1 mL heparinized blood was mixed with an equal volume of 0.4% formaldehyde or paraformaldehyde in phosphate-buffered saline (PBS). The mixture, pre-warmed to 37°C, was incubated for 4 minutes at 37°C and then 40 mL of pre-warmed 0.85% (w/v) NH₄Cl in Tris-HCl buffer [Tris(hydroxymethyl)-aminomethan 0.01 mol/L, pH 7.4] was added. The mixture was incubated for another 15 minutes to lyse the erythrocytes. The cells were thereafter centrifuged for 5 minutes at 350 x g at room temperature, and then the supernatant was removed. The cells were washed twice with PBS containing sodium citrate (0.012 mol/L) and human serum albumin (HSA) (0.1%, w/v). Finally, the cells were suspended in PBS with sodium citrate and HSA, diluted to the concentration of 1.7 - 2.5 x 10⁶/mL and kept at 4°C.

Labelling of leukocytes with antibodies to cell surface antigens

Paper I and II. Fifty µL samples of the leukocyte suspension were mixed with 50 µL of optimally titrated mouse monoclonal antibodies (mAb) against
CD11b, CD16, CD18, CD35 (Immunotech S.A., Marseilles, France), CD32, and CD64 (Medarex Inc., Annandale, NJ, USA) and incubated for 30 minutes at 4°C. After incubation, the cells were washed twice with PBS. The cells were subsequently mixed with 50 μL FITC-conjugated rabbit anti-mouse- Ig (Dakopatts A/S, Glostrup, Denmark) and incubated for 30 minutes at 4°C.

**Paper III and IV.** Fifty μL samples of the leukocyte suspension were mixed with 1 or 10 μL of optimally titrated FITC-labelled mouse monoclonal antibodies (mAb) against CD11b, CD64, (Immunotech S.A., Marseilles, France), CD16, CD18 (DakoCytomation Norden A/S, Glostrup, Denmark), CD35 (Serotec Ltd, Oxford, UK), and CD32 (BD Biosciences Pharmingen, San Diego, CA, USA) combined with RPE-labelled anti-CD14 (DakoCytomation) and incubated for 30 minutes at 4°C.

**Paper I-IV.** After incubation, the cells were washed twice with PBS and thereafter diluted with 200 μL PBS with sodium citrate and HSA. Leukocytes were also labelled by an identical procedure with isotype controls for mouse IgG1 (anti-BrdU) and IgG2 (anti-PCNA) (Dakopatts, Glostrup, Denmark) (paper I and II) or negative isotype controls for mouse IgG1 (DakoCytomation). After labeling, the cells were kept at 4°C until analysis.

**Flow cytometry**

Flow cytometric analysis was performed on an EPICS II Profile or EPICS XL-MCL flow cytometer (BeckmanCoulter Inc., Fullerton, CA, USA). Granulocytes were identified based on their forward scatter/side scatter (FSC/SSC) dot-plot profiles. Monocytes were identified based on their forward scatter/side scatter (FSC/SSC) dot-plot profiles and a positive staining with RPE-labelled anti-CD14. The granulocyte and monocyte populations were gated and the FITC-fluorescence measured. The intensity of fluorescence of granulocytes and monocytes was determined and expressed as mean fluorescence intensity (MFI). The specific MFI of the respective cell surface antigens was calculated by subtracting the background MFI obtained with the respective negative isotype control mAb from the value obtained with anti-CD11b, anti-CD16, anti-CD18, anti-CD32, anti-CD35, and anti-CD64. In case of CD64, which is normally not expressed by neutrophils, expression was also given as relative number of positive cells (%), defined as the relative number of cells that expressed CD64 to a higher extent than the negative control.

**Variation of the method**

In blood samples from healthy adults neutrophil cell surface expression of the six receptors, measured as mean specific fluorescence, showed a mean intra-assay coefficient of variation of 6.4% (range 1.2–13.5) (n=10). The
inter-assay variation of the cell surface expression, measured on 20 – 27 samples from 4 persons, showed a mean coefficient of variation of 18.4% (range 13.0-30.4%). Comparison of samples of venous and capillary blood from paired samples from 10 persons indicated a mean coefficient of variation of 17.6% of neutrophil cell surface expression (measured as mean specific fluorescence) of the six receptors.

Statistical analysis
Statistical evaluations were made with the Mann-Whitney U test and Wilcoxon’s matched-pairs test, comparison of more than two groups was performed by Kruskal-Wallis ANOVA and correlation analysis by Spearman’s correlation test. All statistical analyses of the data were performed using CSS Statistica (StatSoft Inc., Tulsa, OK, USA).

HNL (Human neutrophil lipocalin)
For measurement of HNL blood was collected in gel serum separator tubes. Each sample was left to coagulate for 60 - 120 minutes at room temperature before centrifugation. The serum samples were stored at -70°C until analysed.

The samples were all analysed at the same time using a double-antibody radioimmunoassay (RIA) as previously described (111). Each serum sample was incubated with purified HNL protein labeled with the radioactive isotope ¹²⁵I and specific anti-NHL rabbit antibodies. Consequently HNL in each sample and labeled HNL bound competitively to the rabbit anti-HNL antibodies. Sepharose anti-rabbit IgG (sheep) was then used to separate the complexes of anti-HNL and proteins from non-bound HNL, followed by centrifugation and decantation. Thus there was an inverse correlation between the radioactivity in the fraction of rabbit anti-HNL and anti-rabbit IgG complexes and the concentration of HNL in the serum samples. Each serum sample was analysed twice and the result expressed as the mean of the two values.

Serum concentrations of CRP, G-CSF, IFNγ, IL-6 and IL-8
Measurements of the serum levels of G-CSF, IFNγ, IL-6, and IL-8 were performed using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, UK). The detection limits of the respective assays were 20 ng/L (G-CSF), 8 ng/L (IFNγ), 0.7 ng/L (IL-6) and 0.8 ng/L (IL-8). The reference limits as determined by the manufacturer were <39 ng/L (G-CSF), <8 ng/L (IFNγ), <12.5 ng/L (IL-6), and <25 ng/L (IL-8). CRP was measured by an immunonephelometric assay at the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.
Summary of papers

**Paper 1.** Neutrophils from term and preterm newborn infants express the high affinity Fcγ-receptor I (CD64) during bacterial infections

**Background**
The access to a quick and reliable method for early detection of bacterial infections in newborn infants is highly desired.

Three types of Fcγ-receptor are found on neutrophils and monocytes, namely CD64 (FcγRI), CD32 (FcγRII), and CD16 (FcγRIII) (14;57). CD32 and CD16 are normally expressed by neutrophils. In contrast, CD64 is constitutively expressed only by monocytes and, to a very low extent, by neutrophils. During bacterial infections, however, the CD64 expression on neutrophils has been shown to be increased in adult patients (77;78;106). Neutrophils from preterm infants express CD32 and CD16 to a significantly lesser extent than neutrophils from term infants and adults (70).

**Aim**
The aim of this study was to investigate whether expression of CD64 on neutrophils was increased during bacterial infections in preterm and term neonates, in older infants, and in children and to compare the results with those obtained in adults with bacterial infections.

**Methods**
Twelve preterm and term newborn infants with verified or suspected septicaemia were investigated together with 14 infants and children and 6 adult patients all hospitalised because of an acute bacterial infection. Seven non-infected preterm neonates, 14 healthy term neonates, and 26 healthy adults served as controls All blood samples were analysed for the neutrophil expression of CD64 by flow cytometry.

**Results**
Neutrophils from preterm and term neonates, older infants, children, and adults examined during the early phase of a bacterial infection showed a significantly higher expression of CD64 compared with non-infected controls (p<0.001) (Figures 1 and 2). The markedly increased expression of CD64 on neutrophils was evident, both when measured as CD64 positive cells, i.e. the proportion of cells showing a higher fluorescence intensity than the negative control (Figure 1) and as the MFI of the whole neutrophil population (Figure 2), which is related to the number of receptors per cell. The expression of CD64 during bacterial infection was similar (p>0.3) on neutrophils from preterm and term neonates, children and adults.
No significant difference in neutrophil CD64 expression during infection was found between patients with gram-negative and gram-positive infections in any group. Neutrophils from healthy preterm and term neonates showed a significantly (p<0.001) higher expression of CD64 than neutrophils from healthy adults, both when measured as relative amount of CD64 expressing neutrophils and MFI of the whole neutrophil population (Figures 1 and 2). This increased expression was, however, markedly less than the increase noted during bacterial infections (p<0.001).

Discussion
Early diagnosis and treatment are of crucial importance for the outcome of most bacterial infections in neonates. Since CRP, total and differential leukocyte count, and other tests currently used for this purpose have been shown not to be reliable enough in the neonatal period, great efforts have been made to search for new and better diagnostic tools. Determination of the cellular response to cytokines (e.g., by measuring the cell surface expression of certain receptor molecules) might represent one way of disclosing the early stage of the immune response to a bacterial infection. In this study neutrophils from all children and adult patients with bacterial infection and all newborn infants, including the very preterm neonates, with verified or suspected bacterial infection expressed CD64. In addition, neutrophils from even extremely preterm infants expressed this receptor to the same extent as did neutrophils from adult patients.

Conclusion
The results clearly indicate the possible value of measuring the expression of CD64 on neutrophils as an early indicator of bacterial infections, especially in the neonatal period.
Figure 1. The relative amount (%) of CD64 positive granulocytes in preterm and term neonates (filled circles), infants and children (filled triangles), and adults (filled diamonds) with bacterial infection compared with healthy preterm (open circles) and term neonates (open squares), and healthy adults (open diamonds). The median values of each group are indicated.

Figure 2. Granulocyte cell surface expression, measured as mean fluorescence intensity, of CD64 on granulocytes from preterm and term neonates (filled circles), infants and children (filled triangles), and adults (filled diamonds) with bacterial infection compared with healthy preterm (open circles) and term neonates (open squares), and healthy adults (open diamonds). The median values of each group are indicated.
Paper 2. 64 (Fcγ receptor I) cell surface expression on maturing neutrophils from preterm and term newborn infants

Background
The expression of CD64 is increased from an almost negligible to a marked level on neutrophils in patients with bacterial infections, making it a potential candidate for the diagnosis of bacterial infection even in infants (77;78;112). To further elucidate the potential use of CD64 measurement as a marker of infection in neonates, the possible influence on this receptor expression by other factors not directly related to infection must be excluded.

Aim
The aims of the investigation were to study the development of the expression of CD64 with increasing postnatal age (PNA) in preterm infants, and possible changes that may occur during the first few days of life in term infants. Receptor expression on neutrophils from preterm infants was also investigated in relation to RDS, prenatal steroid treatment, preterm rupture of the membranes (PROM), labour itself, GA, birth weight, and PNA. Mainly for comparison, the surface expression of the other two Fcγ receptors (CD16 and CD32,) and some of the complement receptors (CD11b/CD18 and CD35) on neutrophils were also measured.

Methods
Four groups of subjects were studied. Group 1 consisted of 22 preterm newborn infants without obvious clinical signs of infection. In 11 of these infants the receptor expression was followed about once a week up to more than one month of age. Group 2 included 18 healthy term newborn infants. Group 3 was made up of 30 healthy adults and group 4 contained 9 healthy term newborn infants. In group 4 three consecutive analyses were performed: at birth, by day 1, and by days 3–5. Neutrophils from healthy term newborn infants and adults were used as controls. All blood samples were analysed by flow cytometry for the surface expressions on neutrophils and monocytes of CD64, CD16, CD32, CD11b/CD18 and CD35.

Results
1. Neutrophils from preterm newborn healthy infants showed a moderately increased level of CD64 expression that, during their first month of life, was reduced to the level observed on neutrophils from term newborn infants.
2. In term infants the neutrophil expression of CD64 did not change significantly during the study period.
3. In contrast, the neutrophil expression of both CD11b and CD16, two other possible candidates for early diagnosis of bacterial infections, showed sig-
significant changes during the first weeks of life in preterm infants as well as during the first 3-5 days in term infants.

4. No significant relation existed between expression of CD64 or any of the other receptors investigated and RDS, GA, steroid treatment given to the mother before delivery, method of delivery, birth weight, or PROM.

**Conclusion**

These data indicate that markedly increased expression of CD64 on neutrophil granulocytes may be a specific marker of infections in newborn infants. Thus the results support the possibility that measurement of this receptor expression might represent a useful diagnostic tool for early detection of bacterial infections in both preterm and term neonates.
Figure 1. Neutrophil granulocyte cell surface expression of CD16, CD32, and CD64 with increasing postnatal age in preterm infants. In the left panel the expression of CD16, CD32, and CD64 is represented as MFI and in the right panel the expression of CD64 is represented as the relative number of positive cells (%). Median and upper/lower quartile ranges for each 3-day interval are demonstrated. Significant changes compared with the level on the first 3 days of life are indicated.

Figure 2. Neutrophil granulocyte cell surface expression of CD64, CD16, and CD11b of a child followed for 67 days after birth. The child was born after 28 weeks, had a birth weight of 1050 g, and an Apgar score of 1.6. At birth the child had a positive blood culture for E. coli and antibiotic treatment for septicemia was given. The course was then uncomplicated until 58 days after birth when she presented with suspected septicemia, probably caused by E. chloaca. An arrow indicates the time points of suspected septicemia.
Paper 3. Neutrophil CD64 (FcγRI) expression is a specific marker of bacterial infection. A study on the kinetics and the impact of major surgery

Background
An increase in neutrophil CD64 expression is a promising diagnostic marker for early detection of bacterial infections (77;78;112). In order to represent a clinically useful diagnostic tool, this increased expression should last for at least 48 hours. Fever as well as elevated values for CRP and WBC is regularly seen after major surgery. A diagnostic test not affected by the surgical trauma itself would therefore be most valuable for early diagnosis of bacterial infections postoperatively, as well as to avoid unnecessary treatment with antibiotics.

Aim
To investigate the kinetics of the neutrophil CD64 expression during the first 3 days of a bacterial infection in children and adults and to study the possible influence on the neutrophil CD64 expression of a major surgical trauma total hip replacement. In addition, serum concentration of the cytokines G-CSF, IFNγ, IL-6, and IL-8 were measured to further elucidate the mechanisms involved in the up-regulation of CD64 during bacterial infections.

Methods
The study groups consisted of 8 children and 19 adult patients with bacterial infections, 12 patients admitted for total hip replacement for coxarthrosis and 30 healthy adults. Blood samples were collected the first 3 days after admittance or postoperatively for analysis by flow cytometry of the surface expressions on neutrophils and monocytes of CD64, CD16, CD32, CD11b/CD18 and CD35, CRP and WBC. In the adult patients serum levels of G-CSF, IFN-γ, IL-6 and IL-8 were analysed by ELISA.

Results
Although declining somewhat over time, the expression of CD64 on neutrophils from both children and adults with bacterial infections was significantly increased all 3 days after start of treatment (p<0.0001). After surgery the neutrophil expression of CD64 was increased compared with healthy adults (p<0.001). This postoperative increase was, however, significantly less than the increase seen during bacterial infections (p<0.0001). When compared with reference values, a significant increase in serum levels of G-CSF and IL-6 was found day 1 after admittance for bacterial infections as well as 48 hours (G-CSF and IL-6) and 72 hours (IL-8) after surgery.
Conclusions
1. The increased neutrophil expression of CD64 during bacterial infections lasted long enough to render this receptor expression suitable for diagnostic purposes in this aspect as well.
2. The small, and for diagnostic purposes unimportant increase in neutrophil CD64 expression seen after surgery, makes CD64 a promising diagnostic tool for early detection of severe bacterial infections even during the first days after major surgery.
3. Being elevated also after surgery, the increased serum levels of G-CSF and IL-6 observed during bacterial infections cannot explain the increased neutrophil CD64 expression that is caused by the infection. Thus the precise mechanisms regulating this receptor expression are still to be found.

Figure 1. Cell surface expression of CD64 on granulocytes from adult patients and children with bacterial infection and adult patients 48 hours after operation. Significant differences between the three groups are indicated. The 97.5 percentile of the reference group (20% in case of relative number of positive cells and 1.0 in case of relative MFI) is indicated by a dotted line.
**Paper 4.** Cell surface expression of FcγRI (CD64) on neutrophils and monocytes in patients with influenza A, with and without complications

**Background**

The expression of CD64 (FcγRI) on neutrophils is up-regulated during bacterial infections, which makes CD64 a promising diagnostic tool in the diagnosis of acute infections (77;78;112). To further investigate the usefulness of neutrophil CD64 expression as a diagnostic marker for bacterial infections we studied the possible influence on this expression of a common viral infection, Influenza A.

**Aim**

To study the surface expressions of CD64 on neutrophils and monocytes in patients with Influenza A with and without complications and evaluate these as diagnostic tools in comparison with serum levels of HNL. HNL has been shown to be more useful than CRP in the distinction between bacterial and viral infections (113).

**Methods**

Twenty-two patients with Influenza A with or without complications were included and the results compared with those of 29 patients with bacterial infections and 29 healthy controls. The expression of CD64 on neutrophils and monocytes was evaluated by flow cytometry. HNL was assayed by a specific RIA. The serum level of CRP was included in the panel of variables forming the basis for the diagnosis of the diseases and therefore could not be evaluated for comparison.

**Results**

Neutrophil expression of CD64 was increased in Influenza A infection with raised proportion of cells expressing CD64 in complicated as compared with uncomplicated influenza. The expression was significantly higher in bacterial infections as compared with both influenza groups. Serum levels of HNL were raised in all infection groups, but significantly more so in the group with bacterial infection. On the other hand, the HNL serum level could not discriminate between complicated and non-complicated influenza A infection. Receiver operating characteristics (ROC) analysis showed that the neutrophil expression of CD64 and the serum levels of HNL had similar diagnostic power in the discrimination between acute bacterial infections and influenza A. Monocyte expression of CD64 was raised in all infections with no differences between subgroups.
Conclusion
Both neutrophil expression of CD64 and serum levels of HNL are promising assays in the distinction between infections that are caused by bacteria or Influenza A, whereas only CD64 could identify patients with complications to their influenza A infection.

ROC-curve (receiver operating characteristics curve) comparing the sensitivity and specificity of the CD64 expression on neutrophils and the serum levels of HNL in the distinction between acute bacterial infections and Influenza A. No difference between the two variables was found.
Discussion

The role of CD64 in diagnosis of bacterial infection

Despite the impressive progress in care and management of very preterm newborn infants during the last two decades, severe bacterial infections still cause significant mortality and morbidity in these infants. The general management of severely ill septic infants has also made notable progress, and at least some new effective antibiotic drugs have become available. Regardless of these advances, the outcome of neonatal septicemia in the very preterm newborn infants has not improved much during this time period (18;79;114;115). Attempts have been made to support and strengthen the immature immune system of these infants in order to prevent septicemia or enhance the treatment of established infections but hitherto with little success. With this aim IVIG, G-CSF and GM-CSF have all been investigated but beneficial results from these studies have so far been limited to small specific groups, an example of which is the preventive effect of G-CSF on neonatal sepsis that is caused by pre-eclampsia related neutropenia (53;54;116;117;117).

When discussing advanced strategies for prevention and treatment of neonatal infections, it should be remembered that good hygienic measures are, and will always be, the single most important action to prevent infections in the NICUs, and thereby also to reduce mortality and morbidity from severe infections in very preterm neonates.

In an established septicemia the single most important factor for a positive outcome is the early start of effective antibiotic treatment. The difficulties in recognising the early, most often vague and unspecific signs of infections in this age group are well known. Since sufficiently reliable tests for early diagnosis of infections are not available, antibiotic treatment has to be started on wide indications which lead to a significant over-consumption of antibiotics with the inevitable long-term consequence of promoting further antimicrobial resistance. This aspect is especially important today in that few new antibiotic drugs will become available during the next 5 to 10 years because of the present low incitement of pharmaceutical companies to develop new antibiotics.

Several characteristics are wanted to achieve an ideal marker for detection of bacterial infections in the neonatal period (98). First, one has to consider that the very preterm newborn infant has an immune system characterised
not only by its immaturity but also by its varying degree of maturity in various aspects. A good infectious marker should therefore depend on some aspect in the immune or inflammatory system that is well developed already at birth and in very preterm newborn infants. As an example, the reduced capacity for up-regulation of CD11b on the surface of neutrophils from preterm and also from term newborn infants compared with neutrophils from adults makes this receptor expression less suitable as a diagnostic marker in newborn infants (31;48;49;118-120). Although the precise mechanism for the regulation of neutrophil CD64 expression is not fully understood, neither in newborn infants nor in adults, it is clear from the results shown in papers 1 and 2 that neutrophils from even extremely preterm newborn infants during bacterial infections express CD64 to the same extent as neutrophils from adult patients.

A good infectious marker to be used in the neonatal period, as well as later in life, should indicate infection early in its course. Furthermore, it should have a good sensitivity as well as specificity. So far, no single test can be said to fulfill all these prerequisites. For example, the increase in CD11b expression is detectable very early: it takes only minutes from endotoxin challenge until an increased cell surface expression can be measured (101;121). It is also a sensitive test but lack of specificity probably makes it less useful in clinical practice (103;104;122). CRP on the other hand, has a high specificity ranging from 90% to almost 100%. The slow increase in serum level of CRP in response to bacterial infections is, however, reflected in the low sensitivity of this parameter when used for initial evaluation of sepsis in preterm infants, being calculated to approximately 60%, which is far too low to be acceptable in this patient group (80;85;98;123).

In contrast to CD11b, CD64 is not stored intracellularly in neutrophils. Instead, it has to be produced de novo in response to cytokine stimulation. In a study on monkeys using infusion of E. coli to cause septicaemia a markedly increased CD64 expression on neutrophils was recognised 3–6 hours after the start of infusion, indicating that neutrophil CD64 expression is a rather early indicator of severe bacterial infections. An example of this is found among the infants described in paper 1. In two twins born after 28 gestational weeks and who suffered from intestinal perforation, peritonitis, and septicaemia, an increased neutrophil CD64 expression was discovered approximately 24 hours before any sign of infection was suspected clinically (actual values: MFI 10.1 and 90% positive cells for one of the twins and MFI 7.5 and 85% positive cells for the other.) A renewed examination of x-ray pictures taken at the time of blood sampling for CD64 analysis showed free air in the abdomen, clearly indicating that an infection was about to be established.

To be useful as a diagnostic marker of bacterial infections the substance measured must be detectable for a sufficiently long time after the onset of symptoms. IL-6 and G-CSF are both examples of otherwise most valuable
diagnostic candidates as their serum level rises very quickly from almost zero in response to a bacterial infection, but tends to decline so soon that the risk of measuring a “false negative” result makes them little useful, at least as the sole diagnostic test (124-127). Used as a supplement to other parameters, though, they might still be valuable (124).

As shown in paper 1, neutrophil expression of CD64 was increased to a level characteristic of bacterial infection for at least 3–6 days after the infection had become symptomatic. Thus in the aspect of duration the neutrophil CD64 expression fulfils the demand for being a practically useful infectious parameter.

Another aspect of the duration of increased expression or elevated serum level of infectious parameters is their possible use to follow the course of the infection, for evaluating the effect of treatment, and in some situations, for prognostication. Of these, a reliable test for the evaluation of treatment effect is probably the most desired one. As pointed out earlier, it lasts at least 48 hours from the time a blood culture is drawn until a potentially pathogenic bacterium is isolated and its resistance pattern known. During this time interval, and also in the rather common situation in the NICU when no positive blood culture exists in the infant with suspected sepsicaemia, a significant decline in the level of one or more infectious parameters will often be the only laboratory clue available to decide whether the ongoing antibiotic therapy is effective or should be changed. This kind of evaluation will probably be even more important in the future since there are reasons to believe that bacterial resistance to antibiotics will increase rather than decline in the years to come.

CRP is perhaps the most commonly used parameter in the evaluation of antibacterial treatment in newborns as well as in children and adults, although it is not at all optimal for this purpose considering its half-life is as long as 17 hours (83). Nor does PCT, having an even longer T½, seem to be of particular value in this respect (128). Among commonly available parameters the neutrophil count is probably the most reliable one for therapy evaluation, with the exception of special situations such as the presence of neutropenia or leukocytosis of other origin than infections.

As shown in paper 3, an elevated neutrophil CD64 expression is still present at least 3 days after starting treatment of a bacterial infection. This, again, is an advantage when the CD64 expression is used strictly as a diagnostic test in that the possibility to confirm a diagnosis is not restricted to before and just a short time after the introduction of treatment. However, when used as a measurement of the effect of treatment, a quicker decline in the receptor expression would have been desired. According to our results in paper 3, the decline in CD64 expression after initiation of antibiotic treatment seems to equal the reduction in serum concentration of CRP in adult patients with bacterial infection. In the children with bacterial infection, however, the neutrophil CD64 expression declined more rapidly than that of
CRP. It is possible that HNL, having a T½ of just 10 - 20 minutes, is even better to follow the course of an infection (129).

Diagnostic tests for bacterial infections need to have good sensitivity but this makes specificity the most crucial quality of the test. To investigate both the specificity and the sensitivity of a given test, quite a large number of patients have to be investigated. The investigation of specificity, especially in the newborn infants, is often hampered because an undisputed diagnosis of bacterial infection is often missing, which increases even more the number of patients necessary in order to draw meaningful conclusions.

In paper 2 we have chosen a somewhat different approach to elucidate the question as to whether neutrophil CD64 expression is to be regarded as specific for bacterial infections.

Looking into different situations and conditions in the neonatal period that might influence the neutrophil CD64 expression, we found no relation between this receptor expression and prenatal steroid treatment, PROM, GA, PNA, or birth weight. Even more important, we found that the common lung disease RDS itself did not induce neutrophil CD64 expression. Therefore, in a number of the most common situations in the neonatal period in which the question of a concomitant bacterial infection is raised no factor apart from a bacterial infection seems to induce neutrophil CD64 expression. The limited increase in the receptor expression found during the first days of life in preterm infants gradually subsided and repeated measurements thereafter did not reveal any cause of markedly increased neutrophil CD64 expression except for bacterial infection. These results strongly indicate that the neutrophil CD64 expression is a rather specific marker for bacterial infections also in very preterm newborn infants.

Since preterm newborn infants are susceptible to severe bacterial infections and since both morbidity and mortality from septicaemia are high among these infants, it is highly unlikely that a neonatologist would withhold antibiotic treatment in a preterm infant suspected of suffering from an upcoming bacterial infection, despite concomitant negative results of one or more infectious markers. Consequently, in everyday clinical practice in NICUs the most common scenario is that of instituting antibacterial treatment on a rather wide indication when a potentially severe infection is suspected, especially in the most vulnerable neonates, followed by a careful re-evaluation of the need of antibacterial treatment during the next 24–48 hours. The access to a reliable infection marker with a proven high sensitivity as well as a high negative predictive value would indeed facilitate the decision to withdraw the antibiotic treatment in a situation when the clinical suspicion of an infection has declined.

Two recent studies have shown neutrophil CD64 expression to be a sensitive marker for neonatal infections (107;108). The first study found neutrophil CD64 expression to be a sensitive diagnostic marker for late-onset nosocomial infection in very low birth weight infants with a sensitivity of
95% and 97% and a specificity of 97% and 99% at the time of sepsis evaluation and 24 hours later, respectively (107). In the second study CD64 was found to be a sensitive marker for the diagnosis of early-onset sepsis and pneumonia in term newborn infants having a sensitivity of 97% and a negative predictive value of 98% 24 hours after the first suspicion of clinical infection (108). Furthermore, in a newly published review article on diagnostic markers of infection in neonates it was concluded that CD64 is probably one of the most useful markers for diagnosis of late onset nosocomial sepsis (98).

Discussions on possible improvements in early diagnosis of neonatal infections should also include the question of surveillance. Already some years ago elevated serum levels of inflammatory cytokines were found as early as 48 hours before a nosocomial infection in preterm neonates was clinically recognisable (91). Attempts have already been made to try to take advantage of this knowledge. A study using monocyte CD11b expression for surveillance regarding up-coming infections in neonates found that this parameter was able to detect infections at least 24 hours before any clinical sign of infection was perceived (130). The sensitivity of an increased CD11b expression was good, but the specificity was only 50%, which is too low to be useful in clinical practice. As described earlier we found neutrophil CD64 expression to be increased in a pair of twins 24 hours before any clinical sign of infection was apparent. As this receptor expression seems to be a diagnostic infection marker with high sensitivity and specificity it might be a good candidate for future evaluation as a marker for infection surveillance in selected groups of newborn infants.

Laboratory and technical aspects

For an infectious marker to be suitable for use in daily clinical practice in NICUs several laboratory and technical characteristics have to be fulfilled, some of which will be discussed with special reference to neutrophil CD64 expression.

– First, such a test must be possible to perform on a minimal amount of blood sample. The volume required for analysing CD64 is about 50μl of whole blood, a volume that allows the test to be performed at least once daily for a limited time.

– Second, handling and analysing the collected blood sample should be possible to perform within the daily routine work of the laboratory, not requiring any special form of management. Moreover in this respect the neutrophil CD64 expression fulfils the criterion. From our experience, it may last up to 2 hours from the time of sampling until the specimen is taken care of in the laboratory and the first step in the analysing procedure, which is
Neutrophil CD64 expression in adult patients

In addition to being a marker for diagnosis of severe neonatal infections, CD64 could possibly be a valuable marker in other clinical circumstances where today’s diagnostic tools do not fulfill the requirements. One such clinical condition is the diagnosis of bacterial infections during the first few days after an operation, in which the surgical trauma itself causes fever as well as leucocytosis and an elevated serum level of CRP, making these parameters practically useless for the diagnosis of an up-coming bacterial infection. This situation is to some extent like the one just described in the neonates in that treatment with antibiotics will often have to be instituted on a rather wide indication in order to avoid a more serious infection, but then also with unavoidable over consumption of antibiotics and the consequent risk of further development of antimicrobial resistance. A reliable diagnostic marker not affected by the surgical trauma itself, would be most useful in detecting and treating a post-operative infection early without an undesirable concomitant increase in the total amount of antibiotics prescribed. As described in paper 3 the neutrophil CD64 expression is not increased by the rather extensive surgery total hip replacement to the extent that implicates on its diagnostic usefulness as a marker for bacterial infections. In our study one patient developed a post-operative wound infection, which resulted in a
markedly increased CD64 expression before the wound infection itself was noted. This nicely illustrates that the inflammatory response caused by the surgical trauma itself does not hamper the neutrophil CD64 expression, something which at least theoretically might be possible.

Under certain circumstances early detection of post-operative infections is desirable not only with regard to the patient’s health in general. If, for instance, a patient has been given an artificial hip joint, very early recognition and treatment of an up-coming infection might prevent an otherwise inevitable removal of the prosthesis followed by extensive antibacterial treatment before a new attempt can be made. The same argumentation can be used in other prosthetic surgery situations as well.

The neutrophil CD64 expression has been shown to be a useful marker in distinguishing between acute inflammatory autoimmune disease and systemic infections (109) illustrating that the neutrophil CD64 expression is capable of distinguishing between a bacterial infection and inflammation that is caused by other mechanisms. Further studies should be carried out focusing on the possible advantage of neutrophil CD64 expression for early diagnosis of bacterial infections in clinical situations characterised by non-infectious inflammation, examples of which are extensive traumas, combustion, and pancreatitis.

An example of post-operative septicaemia

In order to illustrate the possible usefulness of the neutrophil CD64 expression for early diagnosis of severe post-operative infections, an example is given in Fig. 1, where simultaneous changes observed in the expression of CD16 and CD11b are illustrated.

The patient, a teenage boy, underwent surgery of both the intestine and the bladder. Antibiotics were administered prophylactic pre- and post-operatively. Day 0 is the day of surgery. On day 1 (24 hours post-surgery) everything seemed normal.

The expression of the neutrophil cell surface receptors, however, was clearly signaling a severe infection. First the expression of CD64 had started to increase and its MFI level is already well above the 97.5 percentile of the reference group. In addition, a striking reduction of CD16 and a marked elevation of CD11b are noted: all these changes are characteristic of a severe infection (as described in paper 3, no changes in the expression of CD16 and CD11b were noted during the first 3 days after surgery or after start of treatment of a bacterial infection).

Not until 48 hours after start of surgery was it noted that something probably was wrong. A urinary obstruction was first suspected, but excluded by ultrasound. About 56 hours post- surgery, and more than 24 hours after the examination of the neutrophil expression of CD64 and the other surface
receptors had been signaling a severe up-coming infection, a septicaemia was suspected. The patient was then referred to the ICU. The course was complicated some hours later by a heart arrest from which he was successfully resuscitated. In the following days an intestinal perforation was found as a cause of the Gram negative sepsis, from which the patient in the end recovered completely. Serum levels of CRP and WBC were initially elevated but of no help for the diagnosis of septicaemia because of the newly performed surgery.

Cell surface expression of CD64, CD16 and CD11b on granulocytes from a teenage boy during abdominal surgery. The upper 97.5 percentile of the reference group for granulocyte expression of CD64 is indicated by a dashed line. The arrow indicates the time point of suspected sepsis.
Possible biological role of Neutrophil CD64 expression during bacterial infections

The main function of the neutrophil granulocyte is to phagocytose and then kill bacteria and other micro-organisms. To enhance phagocytosis components from the complement system and/or specific antibodies bind to the bacterial surface and then to specific receptors on the neutrophil cell surface, the complement receptors (CR1 and CR3) and the Fcγ-receptors (FcγRI – III), as described earlier. The process of phagocytosis is significantly improved when the bacteria are in this way opsonised, increasing as much as 100-fold or more in efficiency (61;76). Using specific antibodies against CD64 (FcγRI) for stimulation of the neutrophil, increased phagocytosis as well as intra-cellular killing of bacteria are noted (ref). Since CD64 is the only Fcγ-receptor capable of binding immunoglobulin monomeric, it might be speculated that CD64 is of special importance for the neutrophil function under conditions characterised by low concentration of specific immunoglobulins, i. e. during the early phase of a bacterial infection at any age, and especially in preterm newborn infants whose immunoglobulin levels are generally very low. This might explain why the expression of CD64 on neutrophils seems limited in time to the first week or so of a bacterial infection, and why neutrophils from extremely preterm neonates whose neutrophil function and immune function in general are so immature, express this receptor to the same extent or more compared with neutrophils from adult individuals.

In any case, the binding of the Fc portion of a specific immunoglobulin molecule to the CD64 receptor molecules, one by one, is indeed to be considered as a bridge between the innate and specific immune systems.

Regulation of neutrophil CD64 expression

IFN-γ and G-CSF are the only two cytokines known to induce the expression of CD64 on neutrophils. When administrated in vivo in pharmacological doses both cytokines cause a marked neutrophil CD64 expression(72;73;75). In vitro however, only stimulation of neutrophils with IFN-γ causes a CD64 expression, probably illustrating that the in vivo the effect of G-CSF on the CD64 expression is indirect (61;74).

These mechanisms, however, explain only partly the way neutrophil CD64 expression is regulated. First of all, preterm newborn infants, whose neutrophil CD64 expression during bacterial infections tends to be more prominent than later in life, are known to be significantly less effective in producing IFN-γ as compared with adults (131-133). Consequently, according to our present knowledge, one or more other cytokines than IFN-γ must play the dominating role in regulating the neutrophil CD64 expression in...
newborn infants. G-CSF is less likely be the dominating cytokine regarding CD64 expression in newborns, since the level of G-CSF in cord blood, although quite variable, most often is found to be clearly elevated, whereas neutrophil CD64 expression in newborns is only slightly increased, and far from the levels detected during bacterial infections (27;134;135).

Thus the precise mechanisms regulating the neutrophil CD64 expression remain to be revealed. Efforts should be made in the future to further elucidate this aspect of neutrophil function, not only in order to understand theoretically the mechanisms controlling the expression of an infectious marker, but mostly because this might involve aspects of the neonatal immune system of possible interest for the ongoing attempts to enhance the capacity of the very preterm newborn infants to withstand and overcome severe infections.

It should also be mentioned that the mechanisms regulating the CD64 expression on neutrophils and monocytes are, at least partly, different. Earlier studies, including paper 3 in the present series of studies, have found that a surgical trauma induces significantly increased monocyte CD64 expression, while at the same time neutrophil CD64 expression is only slightly increased (136). Furthermore, and in parallel to the increased level of G-CSF in cord blood, the increased serum levels of G-CSF found in adults undergoing surgery do not induce a markedly increased neutrophil CD64 expression. Consequently, the well known increase in G-CSF concentration seen during the early phase of an acute bacterial infection cannot in itself be responsible for the concomitantly markedly increased neutrophil CD64 expression.

The role of neutrophil CD64 expression for differentiating between bacterial and viral infections

Any marker used for diagnosis of bacterial infection should be able to distinguish between bacterial and viral infections otherwise its practical application is rather limited. In a study looking at the expression of different Fcγ-receptors on neutrophils and monocytes a limited increase in the percentage of neutrophils expressing CD64 during viral infections was observed, but no significant increase in the MFI-level (78). In contrast, patients with bacterial infections showed a marked increase in neutrophil CD64 expression, expressed both as MFI values and percentage of CD64 positive cells. In order to further investigate the ability of neutrophil CD64 expression to distinguish between bacterial and viral infections we decided to choose infections with Influenza A virus mostly because it represents a severe viral infection, known to be able to induce a rather strong inflammatory response, often reflected in an increased serum level of CRP. In addition, the diagnosis of Influenza A infection is usually rather easy to verify, either by direct identi-
fication of the virus or by typical symptoms in combination with concomitant epidemiological data. Again however, the lack of gold standard sometimes makes the diagnosis uncertain as to whether a specific patient also suffers from a concomitant bacterial infection. Since the diagnosis of bacterial infection was reached partly by the aid of CRP, the capability of neutrophil CD64 expression to differentiate between bacterial infections and Influenza A infections, with or without complications, could not be compared with CRP. Instead, comparison was made with HNL, a parameter earlier shown to be superior to CRP in distinguishing between bacterial and viral infections (113). In the study described in paper 4 we found the neutrophil CD64 expression and HNL to be equally effective in distinguishing between bacterial infections and Influenza A infections. We therefore conclude that neutrophil CD64 expression should be at least equal to CRP regarding this point, although of course further investigations are required to fully answer this question. Finally we also need to examine infections with different viruses in varying age groups.

Concluding remarks

The need of new and better infectious markers for early detection of severe bacterial infections among neonates, and above all among the most infectious prone very preterm newborn infants, is obvious. However, in many other clinical situations access to a quick and reliable marker not affected by inflammation in general but only by a bacterial infection would be most valuable for early institution of effective antibacterial treatment, and to avoid unnecessary use of antibiotics. The neutrophil CD64 expression fulfils many of the characteristics desirable for such a marker, and could already be put into clinical use (i.e., for confirmation or exclusion of a suspected neonatal sepsicaemia day 1 or 2 after start of treatment, for diagnosis of bacterial infections after major surgery, and in patients suffering from autoimmune diseases). For CD64 to be used in the acute diagnosis of infections, however, further methodological development is necessary.
Conclusions

Neutrophil CD64 expression is probably a specific marker of bacterial infections in preterm and term newborn infants, children and adults.

Neutrophils from even extremely preterm infants express CD64 to the same extent as do neutrophils from children and adults.

Neutrophils from non-infected preterm newborn infants express CD64 to a somewhat higher extent than neutrophils from healthy adults. This moderately increased expression gradually declines and does not reach the levels noted during bacterial infections.

Neutrophil CD64 expression is not affected by the respiratory distress syndrome (RDS) itself nor is it affected by premature rupture of the membranes, gestational age, postnatal age, steroid treatment before delivery, method of delivery or birth weight.

In healthy term newborn infants no change in neutrophil CD64 expression is observed during the first few days of life.

In both children and adults with bacterial infections the neutrophil CD64 expression is significantly increased during the first 3 days after start of antibiotic treatment, although it declines somewhat over time.

Neutrophil CD64 expression is increased during the first few days after major surgery, but not to the extent seen during bacterial infections.

The capacity of neutrophil CD64 expression to distinguish between bacterial infections and Influenza A infection is equal or slightly superior to that of human neutrophil lipocalin (HNL), which in a former study has been shown to be superior to CRP in distinguishing between bacterial and viral infections.
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