Altered Neutrophil Counts at Diagnosis of Invasive Meningococcal Infection in Children

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Background: Invasive meningococcal infections can be devastating. Substantial endotoxemia releases mature and immature neutrophils. Endothelial margination of mature neutrophils may increase the immature-to-total neutrophil ratio (ITR). These changes have not been previously well-described in invasive meningococcal disease.

Methods: Using 2001 to 2011 data from the US Multicenter Meningococcal Surveillance Study, the diagnostic sensitivity and clinical correlates of white blood cell count, absolute neutrophil count (ANC), immature neutrophil count (INC) and ITR were evaluated alone and in combination at the time of diagnosis of invasive meningococcal disease.

Results: Two hundred sixteen patients were evaluated: meningococcemia (65), meningitis (145) and other foci (6). ANC ≤1000/mm^3 or ≥10,000/mm^3 was present in 137 (63%), INC ≥500/mm^3 in 170 (79%) and ITR ≥0.20 in 139 (64%). One or more of these 3 criteria were met in 204 of the 216 (94%). Results were similar for meningococcemia and meningitis subgroups. All 13 cases with mildest disease met 1 or more of the 3 criteria. Eight children presented with ANC <1000/mm^3; 3 of them died and a fourth required partial amputation in all 4 limbs.

Conclusions: Invasive meningococcal disease is characterized by striking abnormalities in ANC, INC and/or ITR. Neutropenia was associated with a poor prognosis. Notably, without INCs, 37% of cases would have been missed. Automated methods not measuring immature white blood cells should be avoided when assessing febrile children. Serious infection should be considered when counts meet any of the 3 criteria.

Key Words: Meningococcemia, meningitis, Neisseria, leukocyte, neutrophil

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Invasive infection with Neisseria meningitidis is a relatively uncommon but life-threatening infection in children. In the United States, there are approximately 2500 cases per year, with a mortality rate of 7–15%. Among survivors, the risk of serious sequelae is approximately 15%. This includes irreversible brain injury, hearing loss and loss of digits or extremities or other tissues due to extensive necrosis.

Early diagnosis and treatment may minimize such outcomes. Meningococcal infection usually progresses rapidly over 8 hours, and in some cases results in death within 12–24 hours after symptom onset. At presentation, the classical finding of petechial or purpuric rash or meningeal signs are often absent. For these reasons, moderately ill children with early meningococcal disease presenting with a nonfocal fever may be sent home with or without antibiotics, often with a diagnosis of “viral syndrome.” At that point, meningococcal infection may not have been strongly considered in the differential diagnosis. Studies have shown that children with meningococcal disease are discharged to home from their initial illness visit in 13–49% of cases. In 1 study, 67% of those sent home did not receive antimicrobials, and these children suffered nearly twice as many complications as those who did.

Rapid progression and severity of disease can be attributed in part to intense stimulation of the host immune response. Large amounts of highly bioactive lipooligosaccharide endotoxin are released in blebs actively shed from the meningococcal outer membrane. Consequently, high levels of circulating lipooligosaccharide endotoxin stimulate cytokine release, which engenders extensive liberation of both mature polymorphonuclear (PMN) and immature neutrophils.

This process also activates binding proteins on the surface of leukocytes and endothelial cells. The interaction of these binding proteins results in neutrophil adherence to the endothelial cell wall of blood vessels, called margination. Immature neutrophils apparently do not marginate as effectively as PMNs and therefore tend to remain in the circulation.

Thus, changes in the peripheral white blood cell (WBC) count during meningococcal disease might include increased circulating PMNs or bands due to narrow release or decreased circulating PMNs as a result of extensive margination and migration into infected tissue. Because bands apparently remain in circulation, an increase in the immature-to-total neutrophil ratio (ITR) may also be identified in the differential count. Changes in other cell lines such as lymphocytes and monocytes in meningococcemia have not been described.

Thus, vigorous inflammatory illnesses such as meningococcemia may increase mature neutrophils (PMNs), decrease mature neutrophils, increase levels of nonegmented neutrophils (mainly bands) or increase the ratio of immature neutrophils to total polymorphonuclear neutrophils. Such findings might therefore be likely in meningococcemia. Previous studies have not fully described these parameters. The present study was undertaken to describe the absolute numbers of mature PMNs, immature neutrophils and the ratio of immature-to-total neutrophils.
Fisher's exact test.

Meningococcemia was defined as a positive blood culture for *N. meningitidis* without meningitis, pneumonia or arthritis. Meningitis was defined by a CSF culture or rapid antigen test positive for *N. meningitidis*, or CSF pleocytosis with >5 WBCs per mm³ together with a positive meningococcal blood culture. A child with a positive culture and a chest radiograph consistent with pneumonia was considered to have meningococcal pneumonia. For each episode of infection, a retrospective questionnaire to document demographic and clinical information, as well as outcome, was obtained. Mildest disease was defined as any case without brain injury, tissue necrosis, amputation, consumptive coagulopathy, requirement for pressors or a ventilator or resulting in death.

WBC count parameters included total WBC count outside the strict normal range for age using established standards. Differential cutoff values were chosen in advance, selecting values that most physicians would find markedly abnormal. These parameters were absolute neutrophil count (ANC) less than or equal to 1000/mm³ or greater than or equal to 10,000/mm³, immature neutrophil count (INC) greater than or equal to 500/mm³ and ITR greater than or equal to 0.20.

Among the 261 patients originally enrolled, 45 (17%) were excluded because a manual differential was not available. Of these, 28 had no differential information, and 17 had only an automated differential, leaving 216 for inclusion in the analysis. For excluded cases, distribution between centers and complication rates were differential, leaving 216 for inclusion in the analysis. For excluded cases, distribution between centers and complication rates were similar to included cases. Dichotomous variables were assessed by Fisher's exact test.

METHODS

The US Multicenter Meningococcal Surveillance Study, a collaboration of 10 children's hospitals, identified all children admitted with invasive disease due to *N. meningitidis*. Isolation of this bacterium from a sterile body site was required for inclusion. Patients were enrolled from January 1, 2001, through December 27, 2011.

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RESULTS

Of the 216 enrollees with invasive meningococcal disease, 65 had meningococcemia, 145 had meningitis, 4 had septic arthritis, and 2 had bactereemic pneumonia, 1 complicated by meningococcal empyema. Ages ranged 1 month to 20 years; 131 (60.6%) were younger than 4 years. Among enrollees, 51 (23.6%) required mechanical ventilation and 15 (6.9%) died.

Neutrophil parameters among these enrollees were as follows: ANC <1000/mm³ or >10,000/mm³ was found in 63.4%, INC ≥500/mm³ in 78.7% and ITR ≥0.20 in 64.4%. Thus the sensitivity of each variable alone was limited. However, one or more of the ANC, INC or ITR was abnormal in 204 of the 216, 94.4% of the cases (Fig. 1 and Table 1). Total WBC count was normal in 33.3% of cases.

Results were similar for subgroups with either meningococcemia or meningitis and were independent of established age. Notably, 1 or more of the ANC or INC or ITR criteria were met for all 13 cases with mildest disease as defined in Methods (13/13, sensitivity 100%). This was not statistically different from the remainder with more severe disease (191/203, 94.0%). The age distribution of the mildest disease group was not statistically different from the remainder of cases.

A low ANC carried poor prognosis. Eight children presented with ANCs <1000/mm³; 3 (37.5%) died, compared with 12 (5.8%) deaths among the remaining 208 with normal or elevated ANC (P = 0.01). One surviving child with ANC <1000/mm³ required partial amputation in all 4 limbs.

### TABLE 1. Sensitivity of Leukocyte Parameters Alone and in Combination

<table>
<thead>
<tr>
<th>Leukocyte Abnormality</th>
<th>Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC abnormal for age</td>
<td>143/216 (66.2)</td>
</tr>
<tr>
<td>ANC &lt;1000/mm³ or ≥10,000/mm³</td>
<td>137/216 (63.4)</td>
</tr>
<tr>
<td>INC ≥500/mm³</td>
<td>170/216 (78.7)</td>
</tr>
<tr>
<td>ITR ≥0.20</td>
<td>139/216 (64.4)</td>
</tr>
<tr>
<td>ANC or INC criteria</td>
<td>202/216 (93.5)</td>
</tr>
<tr>
<td>ANC or ITR criteria</td>
<td>197/216 (91.2)</td>
</tr>
<tr>
<td>INC or ITR criteria</td>
<td>177/216 (81.9)</td>
</tr>
<tr>
<td>ANC or INC or ITR criteria</td>
<td>204/216 (94.4)</td>
</tr>
</tbody>
</table>

DISCUSSION

This study demonstrated robust stimulation of neutrophil kinetics in childhood meningococcal disease. Specifically, marked changes in total neutrophils, band counts and ITR were found. Only 5.6% of cases did not meet any of the 3 study criteria.

It is to be noted that a completely normal total WBC was found in 33% of cases. This has practical importance because it indicates reliance on total WBC alone would have been falsely reassuring. In addition, even slightly abnormal total WBC did not add any additional positive cases to those meeting 1 of the other criteria. The same was true of the ANC, which was normal or only mildly abnormal in 37% of cases. This, too, has practical importance because most automated differential counters in use today report ANC but do not report bands or other immature neutrophils. Thus, the automated count would have been falsely reassuring in 37% of cases.

It seems that children presenting with an acute nonfocal febrile illness are commonly categorized as having a “viral syndrome.” The present study indicates this may be inappropriate if blood counts meet 1 or more of the 3 criteria reported here.

Limitations of this study include the exclusion of 17% of study cases (45/261) because differential counts were not

**FIGURE 1.** Abnormal leukocyte differential counts in children with invasive meningococcal disease. WBC indicates white blood cell count with any abnormality for age; ANC, absolute neutrophil count <1000/mm³ or >10,000/mm³; INC, immature neutrophil count >500/mm³; ITR, immature-to-total neutrophil ratio >0.20.

*p ≤ 0.0001 vs. each of the four individual parameters.
available. In addition, the parent study included only children with meningococcal disease, and there was no control group with other febrile illnesses, so positive predictive value of the criteria could not be determined. Furthermore, differential counts were available only on the day of hospital admission, and not from any earlier visits for the illness. A study including such visits would help further assess the value of these criteria for early diagnosis of meningococcal disease.

A previous study of blood cell counts in children sent home with unsuspected meningococcal disease had a mean band count 2300/mm³ at that time. The utility of the band count in combination with the other 2 parameters used here, however, was not evaluated, and the authors concluded neutrophil counts had little value in the early diagnosis of meningococcal disease. Thus, we believe future studies describing neutrophil counts in meningococcal disease, as well as other pediatric infectious diseases, should analyze all 3 parameters, using either manual counts or newly emerging automated systems that recognize immature WBCs.

In this regard, other serious febrile conditions of infants and children may commonly have similar neutrophil changes, including a left shift. Studies reporting such changes include nonbacterial diseases such as Kawasaki disease and hypoxic bronchiolitis due to respiratory syncytial virus. Certain invasive enteric bacterial infections such as Salmonella, Shigella and Campylobacter typically have a prominent shift to band forms. Occult infant pneumococccemia has been associated with elevated total neutrophil count, but, in contrast to meningococcal disease and invasive enteric bacterial disease, there was no further added predictive value from band counts. Neutrophilia has been associated with acute pyelonephritis but not cystitis. It is not clear whether the degree of abnormality in any of these bacterial infections is as great as that reported here for meningococcal disease. Neutrophilia is also frequent in adenovirus infection. Neither mature nor immature neutrophils are common with normoxemic respiratory syncytial virus and human herpesvirus 6 infections, despite high fever associated with the latter.

SUMMARY

To our knowledge, the present study is the first to assess these 3 neutrophil screening parameters at the time of diagnosis of invasive meningococcal disease. A high correlation with 1 or more of these abnormalities was found, whereas total WBC and total neutrophils alone were frequently normal or unimpressive. Results show intense neutrophil changes compatible with induction of cytokines by high levels of bioactive endotoxic lipooligosaccharide.

IMPLICATIONS

Automated blood cell counts that do not measure bands will frequently be falsely reassuring in children with invasive meningococcal disease. Determination of ANC, INC and ITR are to be encouraged whenever blood cell counts are ordered for assessment of children with fever, to aid in the early identification of invasive bacterial infections including meningococcal disease. Serious bacterial infection should be considered in febrile children when WBC counts meet 1 or more of the 3 criteria. Future studies describing neutrophil counts in meningococcal or other pediatric infectious or inflammatory conditions should consider all 3 parameters in the analysis.

REFERENCES