The study has demonstrated that when a clinician asks a specific question and provides clinical information, the pathologist can be of assistance in the majority of cases. The major factors which contribute to the pathologist's inability to provide a useful report appear to be the inadequacy of the specimen and lack of clinical and menstrual information. Thus, it would seem that the responsibility lies with the clinician to provide this information and to supply suitable material if he wishes to gain the most from the histopathology service. Providing that there is close cooperation between clinicians and pathologists, dilatation and curettage, with subsequent histopathological assessment of the endometrium, can be valuable. However, care should be taken regarding over-use or misuse of the procedure, and its cost-effectiveness should be considered in each individual case.

Possibly, more attention should be paid to Dallenbach-Hellweg's basic reminder that before curettage is performed, the clinician should ask himself two questions, namely: will curettage contribute to the diagnosis? and what dangers are there in the procedure? To this might be added: what can I tell the pathologist about the patient?

REFERENCES

Serum Complement Concentrations, Nutritional Status and the Outcome of Measles and Measles Pneumonia

ANN ORREN, A. KIPPS, E. B. DOWDLE, SUZANNA SHEARING, EBBA FALLS

SUMMARY
A prospective study of children with measles has shown a significant association between malnutrition and a poor prognosis. Levels of a number of complement components bore no relationship to the severity of the disease or to its prognosis. Some of the children with acute measles had depressed serum concentrations of factor D, Clq or C3, but complement deficiency does not appear to be implicated in the heightened susceptibility to secondary bacterial and viral infection so commonly found after acute measles.


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Measles, in socially and economically deficient societies, often carries a serious prognosis. Pneumonia, occurring during the acute or postexanthematous phase, is the most frequent cause of the high morbidity and mortality associated with the disease in these communities. The pneumonia is frequently caused by secondary viral infection and is often responsible for extensive damage to pulmonary tissues. Children from these communities also tend to contract measles before the age of 2 years and often before they are even 1 year old.

Although the manner in which adverse social circumstances and infection at an early age interact to influence the clinical course of measles is obscure, it is reasonable to assume that dietary deficiency may aggravate the clinical course of the disease.

Circumstantial evidence in support of this assumption is to be found in the following well-documented associations between measles, host immunity and malnutrition. In the first instance, it is well known that measles, even in well-nourished children, is associated with a period of immunological unresponsiveness. Secondly, there is abundant evidence in the published literature to indicate that protein energy malnutrition (PEM) is associated with a secondary immunodeficiency which may affect cellular, antibody or complement-dependent immune mechanisms. Children
who are hospitalized with measles complications in Cape Town seldom suffer from severe PEM but present in a state of borderline malnutrition. Such children would not be expected to suffer the immune deficiencies described for severe PEM. It is entirely possible, however, that the 'normal' immunosuppression found in measles and the effects of even mild malnutrition act synergistically\(^a\) to compromise host resistance to other infections.

Thirdly, cell-mediated\(^b\) and humoral\(^c\) immune mechanisms are probably involved in the elimination of measles virus from infected individuals. While the requirement for an intact complement system for recovery has not been defined, it has been suggested by Charlesworth et al.\(^d\) that pathological activation of complement occurs in approximately 40% of normal children with uncomplicated measles. Perrin et al.\(^e\) demonstrated that the alternative complement pathway was responsible for lysing, \textit{in vitro}, sensitized measles-infected cells. Hicks et al.\(^f\) found maximum lysis if both alternative and classic pathways were intact. \textit{In vitro} addition of free measles virus to antibody and complement leads to the consumption of classic components.\(^g\) Some viruses need only antibody and early classic components for neutralization.\(^h\) Despite the \textit{in vitro} experiments demonstrating the importance of the alternative pathway in measles, little is known of the functional activity of the alternative pathway components during the course of the disease.

In this article we report the results of a prospective study in which we have correlated the nutritional status of children with measles with the eventual outcome of the disease. We have also sought to establish whether measles in children from local communities is associated with pathological depletion of classic or alternative pathway complement components that might be related to nutritional status, or might influence the prognosis.

**SUBJECTS AND METHODS**

**Patients**

The study included 45 children with measles of sufficient severity to warrant admission to the Cape Town City Hospital for Infectious Diseases and 28 children who attended the Red Cross War Memorial Children's Hospital as outpatients. The sample comprised 49 Coloured children and 24 Black children; 40 (55\%) were girls. The age range was 6 - 57 months and 48 (68\%) were less than 2 years old. Children at the City Hospital were examined 12 - 36 hours after admission and after any dehydration had been corrected; most of them were receiving antibiotics. One child had cerebral palsy and 1 had coarctation of the aorta with ventricular septal defect. Other than malnutrition, no diseases that might have been responsible for hypoalbuminaemia were diagnosed.

All children were investigated on day 0 - 5 of the measles rash. Whenever possible, children were re-investigated approximately 3 months after measles, when they were examined clinically and radiologically for evidence of residual or recurrent pulmonary lesions.

The gross nutritional status of each child was evaluated on the basis of the \textit{Wellcome} system\(^i\) using expected weight for age (EWA) according to the Harvard standards.\(^j\) In order to include cases of borderline malnutrition, children with low serum protein (LSP) levels, that is, serum albumin concentrations below 30 g/l or serum transferrin concentrations below 2 g/l, were classified as malnourished (Table I). Serum albumin concentrations were obtained from the results of total serum protein determinations (biuret method) and serum electrophoresis (Beckman microzone system). Transferrin levels were measured by radial immunodiffusion\(^k\) on Hyland (Costa Mesa, Calif.) plates. Forty-eight per cent of malnourished children were classified on the basis of LSP alone (group A, Table I); the remainder had EWA \(\leq 80\%\) with or without LSP (group B). At presentation 2 children were marasmic; kwashiorkor developed in 4 patients during the course of measles.

Control serum samples for complement assays were obtained from healthy adult blood donors.

**TABLE I. NUTRITIONAL STATUS OF CHILDREN PARTICIPATING IN THE STUDY**

<table>
<thead>
<tr>
<th>EWA(&gt;80%)</th>
<th>EWA(\leq80%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>serum alb.</td>
<td>serum alb.</td>
</tr>
<tr>
<td>and trans.</td>
<td>and trans.</td>
</tr>
<tr>
<td>levels(^*)</td>
<td>levels(^*)</td>
</tr>
<tr>
<td>Nutritional</td>
<td>LSP(^t)</td>
</tr>
<tr>
<td>state</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>A</td>
</tr>
<tr>
<td>City Hospital patients</td>
<td>10</td>
</tr>
<tr>
<td>Red Cross Hospital patients</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
</tr>
</tbody>
</table>

\(^*\) Serum albumin \(\geq 30\ g/l\) and serum transferrin \(\geq 2\ g/l\)

\(^t\) Low serum proteins — serum albumin \(< 30\ g/l\) and/or serum transferrin \(< 2\ g/l\)

EWA = expected weight for age; LSP = low serum protein.

**Complement Assays**

Serum was obtained from blood samples that had been allowed to clot at room temperature for 2 hours. Samples not assayed immediately were stored in aliquots at \(-80^\circ C\) or in liquid nitrogen. Factor B and factor D activities were measured by the radial haemolytic diffusion assay of Martin \textit{et al.}\(^m\) Serum samples were introduced into wells punched in a layer of agarose gel incorporating Mg\(^++\), ethylene-glycol tetracetic acid (EGTA), guinea-pig erythrocytes and factor B- or factor D-free serum. Plates were incubated at \(4^\circ C\) for 17 hours followed by 5 - 7 hours at \(31^\circ C\). Areas of haemolysis were measured.

Complement components C1q, C1s, C5 and C9 were assayed by radial immunodiffusion\(^n\) in plates prepared by incorporating appropriate dilutions of anti-C5 (Meloy, Springfield, Va) anti-C1q, anti-C1s or anti-C9 (Behringwerke, Marburg, Germany) in buffered 1\% agarose; all plates contained a final concentration of 0.005M ethylenediamine-tetra-acetate (EDTA). C3 and C4 were assayed on Behringwerke M-Partigen plates.
A pool of serum obtained from 50 healthy adult blood donors was distributed in small samples and stored at −80°C. This serum pool served as a normal standard for all complement assays and values for all test samples were expressed as percentages of the concentration in the pooled standard serum. A control serum sample was included in each assay.

**Statistical Tests**

Appropriate non-parametric statistical tests described by Siegel\(^7\) were used as indicated.

**RESULTS**

**Influence of Nutritional Status and Age on the Course of the Disease**

Clinical and radiological examinations conducted 3 months after measles, and records of hospital attendances during the 2-6-month period following measles, were used to assess the clinical outcome of the disease. Of the 51 children for whom data were available, 2 had died; both had had severe respiratory disease at the time of death. Fourteen children either had pneumonia (12) or radiological evidence of chronic pulmonary disease (2) 3 months after measles; these 14 were classified together in the pneumonia group. Four children developed tuberculosis; in 1 this was diagnosed in the prodromal period and in 3 in the immediate postmeasles period. Three months after measles, bronchitis was the most frequent complication.

The results of these follow-up studies in City Hospital and Red Cross Hospital children are given in Table II. The results of the analysis of these data are shown in Table III. Children who had had pneumonia or severe recurrent bronchitis in the 6 months preceding measles were omitted from the analysis. Malnourished children were significantly more liable to death or serious respiratory disease than were adequately nourished children, but bronchitis occurred with equal frequency in both groups. Children under 1 year of age were not significantly more vulnerable than older children. There was a tendency for children admitted to hospital with measles to have a poor prognosis, but this was only marginally significant.

**Complement Concentrations during Acute Measles**

Most of the children had pneumonia during the acute stage of measles and approximately two-thirds were to some extent malnourished (Table I). It follows that any tendency towards abnormal complement concentrations could not necessarily be ascribed to measles alone. The children were therefore arranged into three groups: (i) controls — adequately nourished children with acute measles who did not require hospitalization at the time of investigation; (ii) adequately nourished children with measles, complicated by pneumonia; and (iii) malnourished children with measles.

**TABLE II. CLINICAL CONDITION OF 71 CHILDREN AT THE TIME OF EXAMINATION APPROXIMATELY 3 MONTHS AFTER MEASLES**

<table>
<thead>
<tr>
<th>City Hospital</th>
<th>Died</th>
<th>Tuberculosis</th>
<th>Pneumonia</th>
<th>Bronchitis</th>
<th>Clear</th>
<th>Withdrew</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequately nourished</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2†</td>
<td>4*</td>
<td>1</td>
</tr>
<tr>
<td>Malnourished (group A/group B)</td>
<td>2 (2/0)</td>
<td>2 (0/2)</td>
<td>10† (6/4)</td>
<td>7† (2/5)</td>
<td>1 (1/0)</td>
<td>13 (6/7)</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Red Cross Hospital</td>
<td>0</td>
<td>0</td>
<td>3†</td>
<td>6</td>
<td>6†</td>
<td>2</td>
</tr>
<tr>
<td>Adequately nourished</td>
<td>0</td>
<td>0</td>
<td>1* (0/1)</td>
<td>2 (1/1)</td>
<td>3* (3/0)</td>
<td>4† (0/4)</td>
</tr>
<tr>
<td>Malnourished (group A/group B)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Age &lt; 1 year</td>
<td>0</td>
<td>0</td>
<td>6*</td>
<td>6†</td>
<td>3**</td>
<td>5</td>
</tr>
<tr>
<td>Age ≥ 1 year</td>
<td>2</td>
<td>4</td>
<td>8*</td>
<td>11</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Total both hospitals</td>
<td>2</td>
<td>4</td>
<td>14</td>
<td>17</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>

Each * or † represents a child who suffered from pneumonia or recurrent bronchitis respectively in the 6 months preceding measles and who was excluded from the analysis shown in Table III. Each † represents a child who, although not requiring hospitalization, at the time of original investigation, was hospitalized during measles or in the immediate postmeasles period.

**TABLE III. ANALYSIS OF THE RELATIONSHIP OF MALNUTRITION, MEASLES BEFORE 1 YEAR OF AGE AND SEVERE MEASLES REQUIRING HOSPITALIZATION, WITH THE CLINICAL CONDITION OF CHILDREN 3 MONTHS AFTER MEASLES**

<table>
<thead>
<tr>
<th>Status 3 months after measles</th>
<th>Malnourished</th>
<th>&lt;1 year</th>
<th>Hospitalized</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Pneumonia or died</td>
<td>79% (11)*</td>
<td>36% (5)†</td>
<td>86% (12)†</td>
</tr>
<tr>
<td>II. Bronchitis</td>
<td>53% (8)‡</td>
<td>27% (4)‡</td>
<td>47% (7)‡</td>
</tr>
<tr>
<td>III. Chest clear</td>
<td>25% (3)</td>
<td>17% (2)</td>
<td>38% (5)</td>
</tr>
</tbody>
</table>

* Significant difference from III (P = 0.01).
† Marginally significant difference from III (P = 0.05).
‡ No significant difference from III.
Significance levels obtained from the Table of critical values in the Fisher test.\(^2\)
Complement profiles for these three groups of children and for the malnourished group 3 months after measles are shown in Figs 1 - 4. The data obtained from all adequately nourished children 3 months after measles were used to define the reference levels. Levels were regarded as 'depressed' if they were more than 2 standard deviations below the reference mean. The figures also show results obtained for healthy adults. All available data are shown, but 2 children died and a number were unavailable for follow-up.

Fig. 1. Profiles of factor D and C1q concentrations in the serum of adequately nourished children with uncomplicated acute measles (●); adequately nourished children with measles and pneumonia (▲); malnourished children with measles EWA ≤ 80% (○) and LSP only (▲); malnourished children 3 months after measles (□); and healthy adult controls (▼). Means ± SD for adequately nourished children 3 months after measles are indicated by the horizontal lines.

Fig. 2. Profiles of C3 and C5 concentrations in children with measles, in malnourished children 3 months after measles and in healthy adults. For key see Fig. 1.

Fig. 3. Profiles of C1s and C4 concentrations in children with measles, in malnourished children 3 months after measles and in healthy adults. For key see Fig. 1.

Fig. 4. Profiles of factor B and C9 concentrations in children with measles, in malnourished children 3 months after measles and in healthy adults. For key see Fig. 1.

In summary, the results bear emphasis in the following respects:

Considering the grouped data, there was a tendency for factor D, C1q and C3 levels to be lower during the acute
stage of measles than in the same children after recovery. The trend was not significant for adequately nourished children but in the malnourished group it was highly significant in all cases (P<0.005, Mann-Whitney U test). Initial factor D levels in the malnourished group were significantly (P<0.001, Mann-Whitney U test) lower than those in the control group. Factor D levels were equally depressed in malnourished children with low EWA or with LSP only. The fact that the low levels were due to an interaction of measles and malnutrition can be seen from Fig. 1. Except for a few children with overt PEM, those in the malnourished group received no specific dietary therapy and their expected weights for age during the acute stage of measles (mean 82.7%) and after recovery (mean 84.2%) showed no significant change. Despite this lack of improvement in nutritional status, serum factor D activity returned to normal after recovery. If, instead of analysing the grouped data, one considers the individual results, it is apparent that not all children with measles had low factor D, Clq or C3 levels, and that group tendencies could be accounted for by a proportion of the children having depressed levels. Twenty-five out of a total of 66 children tested had depressed factor D levels, 13 out of 57 had depressed Clq levels and 11 out of 65 had depressed C3 levels. Individual serum complement profiles did not show selective depletion of early classic or alternative pathway complement components that would have indicated predominant activation of either of these pathways in vivo. In cases where there was depression of a particular component it was not consistently associated with depressed concentrations of other components. Cls and C5 levels were similar in all groups of children and the adult control group.

Factor B, C4 and C9 levels tended to be high in all groups of measles children compared with those in normal adults. However, high factor B levels and, less frequently, high C9 levels were found in some of the children 3 months after measles. The range of C4 levels was wide and despite the tendency to high C4 levels in acute-stage measles, some children had relatively low levels.

Relationship Between Complement Concentration and Severity and Prognosis of the Disease

The severity of measles or its complications in City Hospital children was assessed from the duration of hospitalization, the median length of stay in the measles ward being 10 days. Children were grouped into: (i) those staying less than 10 days; and (ii) those who stayed 10 days or longer, or who were transferred or readmitted because of postmeasles pneumonia, or who died. In no instance was there a significant difference in the levels of a particular component between groups (i) and (ii).

The influence of complement concentrations on the prognosis of measles was examined by comparing results obtained during acute-stage measles in children grouped according to their clinical status 3 months later (as in Table III). Children were grouped into: I — those with pneumonia, or who had died; II — those with bronchitis; and III — those with clear chests. Fig. 5 shows the results for factor D concentrations. Similar analyses were carried out for the other complement components. There was no evidence that the concentrations of any of the components were related to the outcome of the disease.

**Fig. 5. Profiles of factor D concentrations at the time of acute measles in children who at 3 months after measles had pneumonia or had died (△); had bronchitis (○); and had a clear respiratory system (◎). There is no significant difference in the factor D concentrations between any of the groups (Mann-Whitney U test).**

**DISCUSSION**

In Africa measles is often a very serious disease, particularly in the lower socio-economic groups. Most authors have dealt with the immediate outcome of measles or postmeasles pneumonia. In this article we have attempted to examine the prognosis of measles not only in terms of mortality but also in terms of clinical conditions in the few months after "recovery". The results confirm the experience of many clinicians with regard to the high incidence of respiratory complications in this period and demonstrate a significant association between poor prognosis and malnutrition (Table III).

Several factors may explain the relatively serious outcome of measles in South Africa. The children in the present investigation were younger than most children in Western countries who contract measles. Age may be an important prognostic factor that was not apparent in this study owing to the relatively narrow age range of the children studied. Wesley et al. suggest that chronic pulmonary infection tends to occur in malnourished children because their initial inadequate immunological response to infection results in inefficient elimination of pathogens and
in tissue damage; superadded viral infection tends to lead to further immunosuppression. On the other hand, Coovadia et al. found marked immunosuppression during measles but not in children with chronic postmeasles chest disease. Viral postmeasles pneumonia is responsible for serious non-bacterial bronchiolar and interstitial necrosis, but it is not possible to conclude whether damage to the upper and lower respiratory tracts or a protracted state of immunodeficiency, or an interaction of the two, was responsible for the high incidence of respiratory complications observed in this study.

It may be questioned whether serum albumin and transferrin levels are good indices of malnutrition, particularly during acute infection. The electrophoretic patterns of the serum proteins of the children all showed reactive profiles. Chandra claims that during measles there is diversion from albumin synthesis to globulin synthesis. The hypo-albuminaemia found in many children in the present study probably reflects the inability of the children to maintain levels in the normal range during times of stress. Borderline malnutrition is obviously difficult to define and most workers have dealt with classifications of overt PEM. McLaren et al. used weight, serum albumin concentrations and clinical data to establish grades of malnutrition. McFarlane et al. reported that serum transferrin levels correlate with the degree of malnutrition. On the other hand, Roode et al. found serum transferrin concentrations of no value as a sensitive test for the detection of borderline malnutrition in a group of apparently healthy children. It may be that serum albumin and transferrin measurements are of most value as indices of malnutrition in severely affected children or in children stressed with an infectious disease. Certainly in the present study the group of children classified as malnourished on the basis of biochemical data alone had the same high incidence of respiratory complications as those children with low EWA (Table II).

In many of the children C4 and C9 behaved as acute-phase reactants in that serum levels were elevated during acute measles when compared with those found 3 months later. The high levels of factor B found during measles are difficult to interpret because levels remained high in recovered children. Factor B has been reported as an acute-phase reactant. Children returning for examination 3 months after measles often suffered from some form of respiratory tract infection and this may explain the elevated levels. On the other hand, levels may normally be elevated in young children. Charlesworth et al. report that some children with measles have levels of C4 and factor B that are higher than those of adults, but they too found a wide range of concentrations in normal children.

In vivo consumption of complement during the acute stage of measles is suggested by the present finding of isolated C3, C1q and factor D depression. The tendency for malnourished children to have low factor D levels during acute measles suggests that these children were unable to compensate for its utilization. There appear to be no reports of factor D activity in measles. Charlesworth et al. interpreted their data as indicating that there was activation of the classic complement pathway in some of their patients and activation of the alternative pathway in others. This type of interpretation is difficult to make because it does not allow for variations in complement synthesis and catabolism. Moreover, there is evidence that activation of the whole pathway is not necessary for viral neutralization. Charlesworth et al. found isolated cases of C1q deficiency as described above and this may be an important aspect of the response to measles.

Sirisinha et al. and Olusi et al. studied complement levels in PEM and report depression of all components measured except C4 and C3. In both surveys the children studied were more severely malnourished than the majority of those patients described here. In this study an attempt was made to determine whether a poor prognosis for measles could be attributed to a deficiency of complement components arising from complement depletion acquired during the course of the disease. Deficiencies of C1q, C3 and factor D were found but the C1q and C3 deficiencies were no more striking than those reported for a group of older, less severely affected children. Complement levels bore no relationship to the severity of the disease or to its prognosis. Thus complement deficiency in measles does not appear to be implicated in the decreased resistance to infection so commonly found in these children.

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