Anemia Associated with Acute Infection in Children

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ABSTRACT: **Background:** The pathogenesis of anemia associated with acute infection in children has not been well delineated.
Objectives: To characterize this type of anemia in children with acute infection, mainly in relation to iron status.
Methods: These two cross-sectional studies compared the prevalence and severity of anemia between outpatient febrile children and age-matched non-febrile controls.
Results: In part 1 of the study, children with acute infection (n=58) had a significant decrease in hemoglobin levels compared with 54 non-febrile controls. Mean corpuscular volume (MCV) did not change this association. Moreover, there was no significant difference in MCV, mean cell hemoglobin or red cell distribution width values between the two groups. Regarding part 2, of the 6534 blood counts obtained in community clinics, 229 were defined as "bacterial infection." Chart survey confirmed this diagnosis. White blood cell level was significantly inversely associated with hemoglobin level (r = -0.36, $P < 0.0001$). Anemia was significantly more prevalent among children with bacterial infection compared to those without: 21.4% vs. 14.1% (P = 0.002). Mean values of iron status parameters were all within normal limits.
Conclusions: Acute illness is associated with anemia. The pathogenesis of this anemia does not appear to be associated with disruption of iron metabolism.

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Most children with moderately severe acute infection experience a significant decline in hemoglobin within a week of illness onset [1]. The prevalence of anemia is associated with inflammation and fever duration [2]. Though frequently observed in the clinical setting, anemia associated with acute infection and inflammation is not well characterized.

In a previous study [3] we examined the assumption that a process of hemolysis plays a role in anemia of acute infection. The study population comprised hospitalized febrile pediatric patients who had a positive blood or urine culture. Children admitted to the hospital for elective surgical procedures served as controls. Blood parameters of hemolysis were also investigated. In the 70 patients with pyelonephritis and bacteremia the mean (± SD) hemoglobin on hospital admission was 10.9 ± 1.27 versus 12.1 ± 1.03 g/dl among the controls ($P < 0.0001$). Compared to normal-for-age hemoglobin values as a standard, 42 cases (60%) were identified as anemic. All parameters of hemolysis, namely reticulocytes, bilirubin, lactase dehydrogenase and haptoglobin, were normal.

Children with anemia are often treated with iron-containing medications, though an association between infection-associated anemia and disruption of iron metabolism has not been established. We conducted the present cross-sectional clinical study to investigate the prevalence and nature of anemia associated with acute infection – bacterial and viral – and to determine whether it is associated with markers of iron deficiency.

PATIENTS AND METHODS

PART 1

The present study was designed to examine associations between measures of anemia in children with viral infection vs. those without. This cross-sectional study – approved by the institutional Helsinki Committee – compared blood measures of anemia between children with vs. those without viral infection.

A convenience sample of children aged 3–16 years was recruited from two pediatric outpatient clinics in the Tel Aviv area during the period 3 March to 30 June 2009, inclusive. Children with temperature > 38°C for more than 4 days

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and a tentative diagnosis of "acute viral infection" at the time the complete blood count was performed comprised the population from which cases were identified. Using the CBC results, "viral infection" was defined by the presence of at least one of the following: a) lymphocytosis > 8000/mm^3, b) lymphocytes > 60% of the leukocyte count, or c) lymphocyte + monocyte counts > 60% of leukocyte count. The presence of viral infection defined the subject as a "case."

Control CBCs were obtained from healthy age-matched children whose CBC was measured before elective surgery during the same period.

Anemia was defined as Hgb < 10.5 g/dl in children aged 0–2 years, < 11.5 g/dl in children aged 2–11 years, < 12 g/dl in females aged 12–18 years, and < 13 g/dl in males aged 12–18 years [4].

EXCLUSION CRITERIA

Children with the following characteristics were excluded from the present study: a) history of anemia prior to the onset of the febrile illness, b) treatment with iron-containing medications/supplements, and c) blood transfusion in the 6 months prior to the study.

PROCEDURES

A CBC was obtained from every child with fever of 96 hour duration. Demographic variables, disease characteristics and CBC results were recorded.

PART 2

The present study was undertaken to characterize the association between bacterial infection and anemia in a pediatric population. The study was approved by the Ethics Committees of Wolfson Medical Center and Maccabi Health Services, Israel.

All complete blood counts performed in children aged 0–16 years from 1 January to 31 December 2008 at Maccabi Health Services were included in the present analysis. The following measures were included in the CBC: hemoglobin, hematocrit, red blood cells, white blood cells, mean platelet volume, mean corpuscular volume, mean cell hemoglobin, mean cell hemoglobin concentration, red cell distribution width, platelet count, and % lymphocytes, eosinophils, neutrophils, monocytes, and basophils.

Subjects were categorized according to CBC results into one of two groups: "Bacterial infection," defined as WBC > 15,000 *and* absolute neutrophil count > 10,000, or "Normal," defined as WBC 4000–12,000 and absolute neutrophil count 3000–7000 and lymphocyte count 5000–7000.

Medical records of patients with CBC classified as bacterial infection were evaluated to confirm the diagnosis. Anemia was defined as Hgb < 10.5 g/dl in children aged 0–2 years, < 11.5 g/dl in children aged 2–11 years, < 12 g/dl in females aged 12–18 years, and < 13 g/dl in males aged 12–18 years) [4].

CBC = complete blood count
Hgb = hemoglobin
WBC = white blood cells

STATISTICAL ANALYSIS

Analysis of data was carried out using SPSS 11.0 statistical analysis software (SPSS Inc., Chicago, IL, USA). Continuous variables are described as mean ± standard deviation. Normality of distribution of continuous variables was assessed using the Kolmogorov-Smirnov test (cutoff at $P = 0.01$). All CBC variables and age at time of CBC had distributions significantly deviating from normal and were compared by CBC category (normal vs. bacterial infection) using the Mann-Whitney U test. A model of bacterial infection was developed using logistic regression analysis and odds ratios were estimated with 95% confidence intervals. Categorical variables were compared between cases and controls using the chi-square test. Associations between continuous variables were described by calculating the Spearman rho correlation coefficient. All tests were two sided and considered significant at $P < 0.05$.

RESULTS

PART 1

There were 58 cases (age 6.3 years, range 2.7–16.3) with viral infection. The control group included 54 children aged 6.7 (2.2–16.0) years. Anemia was identified in 9/58 (15.5%) of the cases, compared to 1/54 controls (1.9%) ($P = 0.017$). There was a significantly greater proportion of females among the controls than among the cases: 64.2% vs. 43.1% ($P = 0.03$). The symptoms of the patients are presented in Table 1.

Levels of Hgb, Hct and RBC were significantly decreased in cases compared to controls. Additionally, compared to controls, cases had significantly higher monocytes and % monocytes [Table 2]. Hgb was significantly positively associated with % lymphocytes ($r = 0.21$, $P = 0.03$) and age ($r = 0.56$, $P < 0.0001$), and marginally inversely associated with percent neutrophils ($r = -0.16$, $P = 0.095$) and neutrophil count ($r = -0.17$, $P = 0.07$). All parameters of iron deficiency, namely MCV, MCH and RDW were similar in both groups [Table 2].

PART 2

A total of 6534 CBCs were reviewed, of which 230 (3.5%) met the criteria for classification as "bacterial infection." With the exception of a single patient diagnosed with dehydration, all bacterial infection patients had a diagnosis of "fever" at the time the CBC was taken. The dehydrated patient was omitted from the analysis, leaving 229 patients in the bacterial infection group.

Hct = hematocrit
RBC = red blood cells
MCV = mean corpuscular volume
MCH = mean cell hemoglobin
RDW = red cell distribution width

Table 1. Symptoms in 58 children with febrile illness (some patients had more than one symptom)

No. of children	Symptoms
40	Cough
29	Sniffles
20	Sore throat
9	Myalgia
8	Weakness
3	Diarrhea
1	Eye infection
1	Vomiting

Table 2. Complete blood count characteristics of study populations

	Controls (n=58)		Cases (n=54)		
	Mean	SD	Mean	SD	*P* value
Hgb (g/dl)	12.1	1.0	10.9	1.3	< 0.0001
Hct (%)	36.1	2.9	32.4	3.5	< 0.0001
RBC (10^9/L)	4.6	0.5	4.2	0.5	< 0.0001
MCV (fl)	79.1	8.1	78.1	5.3	0.445
MCH (pg)	26.7	3.0	26.4	2.0	0.513
RDW (%)	14.0	1.7	14.7	2.1	0.054
WBC (10^3/ml)	8788	2643	16117	8951	< 0.0001
Platelets (10^6/ml)	31,5066	81,951	382,434	142,140	0.005

Hgb = hemoglobin, Hct = hematocrit, RBC = red blood cells, MCV = mean corpuscular volume, MCH = mean cell hemoglobin, RDW = red cell distribution width, WBC = white blood cells

Children with bacterial infection were significantly younger than those with normal CBCs: 2.6 ± 3.8 vs. 9.2 ± 7.1 ($P < 0.0001$). Anemia prevalence and CBC findings are presented by bacterial infection status in Table 1. A 33.2% relative increase in anemia was observed in children with bacterial infection vs. children without: 21.4% vs. 14.3% ($P = 0.003$). As shown in the table, most CBC measures were significantly lower in subjects with vs. without bacterial infection, including % lymphocytes and % basophils, though the absolute counts of these measures were significantly higher in patients with vs. without bacterial infection. Relative to patients with normal CBC, those with bacterial infection had significantly increased WBC, MCHC, RDW, platelet count, neutrophil count and % neutrophils, and monocyte count.

MCHC = mean cell hemoglobin concentration

Table 3. Complete blood count characteristics of study population by bacterial infection status

Measure	Normal	Bacterial infection	*P* value
Prevalent anemia (%)	14.3	21.4	0.003
Hgb (g/dl)	12.6 ± 1.7	11.6 ± 1.2	< 0.0001
Hct (%)	37.9 ± 4.7	34.6 ± 3.3	< 0.0001
RBC (10^9/L)	4.7 ± 0.5	4.6 ± 0.39	< 0.0001
WBC (10^3/ml)	8.5 ± 3.4	21.4 ± 5.5	< 0.0001
MPV (fl)	10.2 ± 1.7	9.2 ± 1.7	< 0.0001
MCV (fl)	80.1 ± 7.8	76.2 ± 5.8	< 0.0001
MCH (pg)	26.6 ± 2.8	25.6 ± 2.2	< 0.0001
MCHC (g/dl)	33.1 ± 2.1	33.6 ± 1.0	< 0.0001
RDW (%)	13.9 ± 4.3	14.5 ± 1.6	< 0.0001
Platelets (10^6/ml)	277 ± 82	342 ± 112	< 0.0001
Lymphocyte count (10^3/ml)	3.4 ± 1.7	5.1 ± 2.3	< 0.0001
% Lymphocytes	40.9 ± 13.3	23.8 ± 8.9	< 0.0001
Eosinophil count (10^3/ml)	.22±.22	.11± 0.16	< 0.0001
% Eosinophils	2.7 ± 2.5	.54 ± 0.81	< 0.0001
Neutrophil count (10^3/ml)	3.9 ± 2.2	14.3 ± 4.8	< 0.0001
% Neutrophils	46.1 ± 14.2	66.3 ± 10.1	< 0.0001
Monocyte count (10^3/ml)	0.78 ± 0.44	1.9 ± 0.98	< 0.0001
% Monocytes	9.2 ± 3.1	9.1 ± 3.8	0.87
Basophil count (10^3/ml)	0.04 ± 0.04	0.06 ± 0.04	< 0.0001
% Basophils	0.47 ± 0.38	0.28 ± 0.19	< 0.0001

Hgb = hemoglobin, Hct = hematocrit, RBC = red blood cells, WBC = white blood cells, MPV = mean platelet volume, MCV = mean corpuscular volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration, RDW = red cell distribution width

Hgb was significantly inversely correlated with WBC ($r = -0.36$, $P < 0.0001$), % lymphocytes ($r = -0.17$, $P < 0.0001$), absolute lymphocyte count ($r = -0.4$, $P < 0.0001$) and absolute neutrophil count ($r = -0.1$, $P < 0.0001$), and was significantly positively correlated with % neutrophils ($r = 0.2$, $P < 0.001$). Children with "bacterial infection" had statistically more neutrophils and less percent lymphocytes than the controls [Table 3].

Bacterial infection emerged as a significant predictor of anemia: odds ratio 1.56, 95% confidence interval 1.09–2.20 ($P = 0.01$), indicating that the presence of bacterial infection increased the odds of prevalent anemia by 56%, even after controlling for age and MCV. Age was also positively associated with anemia, such that each 1 year increase in age was associated with a relative 8% increase in risk of prevalent anemia (OR 1.08, 95% CI 1.07–1.1, $P < 0.0001$). All parameters of iron status, namely MCV, MCH and RDW, were within normal limits [Table 3].

OD = odds ratio
CI = confidence interval

DISCUSSION

The present study identified a strong inverse association between hemoglobin and WBC, indicating acute infection. Anemia was identified in 9 of 58 patients (15.5%) with viral infection, compared to 1/54 controls (1.9%) ($P = 0.017$). All parameters of iron deficiency, namely MCV, MCH and RDW, were similar in both groups. Similarly, a 33.2% relative increase in anemia was observed in children with vs. without bacterial infection: 21.4% vs. 14.3% ($P = 0.003$). Hgb was significantly inversely correlated with WBC ($r = -0.36$, $P < 0.0001$), % lymphocytes ($r = -0.17$, $P < 0.0001$), absolute lymphocyte count ($r = -0.4$, $P < 0.0001$) and absolute neutrophil count ($r = -0.1$, $P < 0.0001$), and significantly positively correlated with % neutrophils ($r = 0.2$, $P < 0.001$). All parameters of iron status, namely MCV, MCH and RDW, were within normal limits in this group of anemic patients.

In a previous study on this topic [3] we excluded the possibility that a process of hemolysis causes the anemia associated with acute infection. All parameters of hemolysis in 42 pediatric patients admitted for pyelonephritis and bacteremia who were found to be anemic were normal.

The study had several limitations, the most important relating to the selection of the control population. While lack of fever in the first part suggests that the controls were free of viral infection, no other investigation was made to verify this. Thus, the possibility exists that some of the controls had non-febrile viral infections. Additionally, controls were drawn from children sent for a CBC. Children referred for CBC are not representative of all children. Typically, a child referred for a CBC in our health system is under evaluation for some medical issue. Both of these points would act to misclassify some children with viral infections as controls. Further, some of the controls may have had a bacterial infection or other illness that might be associated with anemia. This would bias the estimation of the association between viral infection and anemia towards the null hypotheses; it is therefore likely that differences between children classified as having viral infection and those classified as controls are an underestimation of the true differences between sick and healthy children.

"Acute infection" in the second part was indicated in the medical record, but "bacterial origin" was not confirmed and cultures were not available. Nevertheless, the CBC-based definition of "bacterial infection" was used consistently and systematically in the cohort, and if misclassification occurred it was likely non-differential, in which case our estimation of the association between Hgb and bacterial infection would be an underestimation.

Both viral infection and "bacterial infection" were associated with increased prevalence of anemia. This is consistent with our previous report, in which 60% of hospitalized children with fever and positive blood culture had new-onset anemia [3].

The decline in Hgb observed in children with acute infection was rapid, and while this might suggest blood loss, this explanation is not relevant in our study population. Hemodilution is yet another explanation consistent with a rapid drop in Hgb levels, but since our subjects were not hospitalized this is not applicable. A defect in erythropoiesis or abnormal iron metabolism cannot explain our observations, because red cells live for 120 days and any aberration in erythropoiesis would be manifested in a more gradual Hgb decline. It has been suggested that decreased red blood cell deformability leads to increased removal of red cells by the reticuloendothelial system [5]. In an animal study where dogs were injected subcutaneously with Freund complete adjuvant [6], hemoglobin level dropped by 25% within a few days [7].

In our study, Hgb was significantly lower in patients with viral infection than in controls. However, there was no significant difference in MCV, MCH and RDW, which implies that anemia following acute infection is not associated with iron metabolism. Similarly, the main parameters of iron status – MCV, MCH and RDW – in the second part of the study are within normal limits [Table 1] and it is unlikely that iron deficiency is responsible for the infection-associated anemia. Unlike the situation in chronic infection where hepcidin plays a major role in the pathogenesis of anemia, the anemia accompanying acute infection does not appear to be associated with iron metabolism. Of course this notion must be directly tested to establish its evidence base. Our study highlights the importance of this step.

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