Pneumococcal Colonization and Pneumococcal Disease in Children with Influenza

Clinical, laboratory and epidemiological features

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We retrospectively studied clinical features of the 2015-2016 paediatric influenza season and the rate of pneumococcal colonization/disease in a reference Romanian infectious diseases institute. Peak influenza activity occurred between weeks 5-10/2016; A viruses initially predominated, switching to B viruses after week 12/2016. Patients' median age was 4.4 years. Patients with influenza A were significantly younger compared with influenza B ($p<0.001$), and required longer hospitalization ($p<0.001$). S. pneumoniae was identified in 5.4% of cases (only influenza A), accounting for 2.1% pneumococcal disease and 3.3% pneumococcal colonization. Patients with S. pneumoniae were younger compared to negative cases ($p=0.164$), presented to the hospital later ($p=0.049$), had higher erythrocyte sedimentation rate (ESR, $p=0.008$), and prolonged hospitalization ($p=0.016$), regardless of whether the strains caused disease or were colonizers. Commonly used inflammation markers may identify the presence of pneumococci (ESR, $p=0.008$) or differentiate between colonization and disease (neutrophil count, $p=0.011$) in children with influenza A.

Keywords: colonization, influenza, otitis media, pneumococcal disease, pneumonia, Streptococcus pneumoniae.

Influenza may associate significant morbidity, particularly in young children [1]. For this reason, influenza vaccination is recommended for all children with ages between 6 months and 6 years old, regardless of underlying comorbidities. The pathogenesis of influenza virus infection (specifically influenza A)[2]. includes an impairment of mucociliary clearance[3] coupled with deficient bacterial phagocytosis [4], leading to a decrease in pulmonary defenses, which is said to *open the gates* for bacterial superinfection, particularly for agents such as *Staphylococcus aureus*, *Streptococcus pneumoniae* or *Haemophilus influenzae*. The latter has significantly dropped in incidence after the introduction of wide-spread vaccination, and the same is true for *S. pneumoniae*. However, pneumococcal vaccination is regrettably not universal and even in vaccine recipients pneumococcal strain replacement may occur. For all these reasons, pneumococci remain major causes of morbidity in patients with influenza, and merit further study to clarify the clinical and epidemiological features of pneumococcal colonization and of pneumococcal disease in patients with influenza.

Experimental part

Material and methods

We performed a retrospective study of the clinical, laboratory and epidemiological features of the 2015-2016 influenza season as reflected by the cases managed in one pediatrics ward of the National Institute for Infectious Diseases Prof. Dr. Matei Bals Bucharest, Romania. We collected epidemiological data for all patients (n=340) admitted for influenza-like illness (as defined by the European Centre for Disease Prevention and Control)[5], and selected 255 cases (75%) to be fully characterized and included in the statistical analysis, based on the following criteria: 1) Cases diagnosed between week 4/2016 and week 16/2016 (the peak influenza activity in Romania); 2) Balanced proportion of influenza A and B cases, consistent with the epidemiology of the entire influenza season 2016 in Romania. These 255 cases were further assessed in terms of clinical and microbiological data on influenza disease characteristics and outcome, and regarding the rate of pneumococcal colonization/disease and its impact on the inflammatory profile expressed as the total white blood cells count (WBC), neutrophil count, erythrocyte sedimentation rate (ESR), fibrinogen and C-reactive protein (CRP). For the purposes of this study, we selected cases positive for influenza A or B through rapid antigen testing. Pneumococcal colonization was defined as positive nasal culture or positive upper respiratory tract secretions on a multianalyte antigen detection test (mariPOC, ArcDia International Oy Ltd, Turku, Finland) not accompanied by any signs of infection, while pneumococcal disease was identified through radiological images suggestive for pneumococcal pneumonia, or evidence of suppurative otitis media with culture-based identification of *S. pneumoniae* from the ear fluid. Due to the retrospective nature of the study, a complete vaccination history could not be obtained for all patients positive for pneumococcus; patients with missing data were considered as not vaccinated for the purposes of this analysis.

We performed the statistical analysis with IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA). We present mean values, standard deviations and results of the paired sample t test for normally-distributed data, and median values plus interquartile ranges (IQR) as well as the results of non-parametric tests for non-normally distributed continuous variables. The effect size for the Mann Whitney U test was calculated as $r = \frac{|Z|}{\sqrt{N}}$ as described in field literature[6].

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Results and discussions

Epidemiology

During the influenza season 2015-2016 (week 53/2015 - week 20/2016), a total number of 340 patients were admitted to the selected pediatric ward for influenza-like illness. Of them, 70.9% had influenza A, 23.8% influenza B, and 5.3% were negative for influenza A or B on rapid antigen testing. In our study population, influenza activity started in week 53/2015, the peak was recorded between weeks 5-10/2016, and the last case presented in week 20/2016, with A viruses predominating early on, and a switch towards B viruses from week 12/2016 onwards (fig. 1). In only 32.5% of cases a clear epidemiologic link was documented: 23.5% had been exposed to siblings with confirmed influenza, 5.9% to unvaccinated parents, 0.8% to unvaccinated grandparents, 1.2% had contact with hospital personnel, 0.8% were exposed to influenza at kindergarten and 0.4% at daycare centers.

For the remainder of the analysis we will be referring to the 255 cases included in the complete statistical analysis. The median age at presentation was 4.4 (2.5, 6.9) years and 12.9% of the patients had prior medical history: 3.5% had had episodes of ear-nose-throat infections, 2.4% repeated respiratory infections, 1.6% history of cardiac disease, 2% atopy including 1.2% asthma, 2% neuro-developmental conditions, 0.8% urinary tract infections, and 0.4% metabolic conditions.

The onset of complications of an otherwise typical influenza-like illness was the main presenting complaint in 74.1% of cases, with 20.4% of patients presenting for myositis, 3.9% for acute otitis media, 3.1% for encephalitis, and another 0.8% for meningismus and intracranial hypertension.

Comparison of cases of influenza A and influenza B

Patients with influenza A were significantly younger compared with those with influenza B ($p<0.001$), with a moderate effect-size ($r=0.3$) (fig. 2). The duration of hospital admission was also significantly longer in cases of influenza A ($p<0.001$, $r=0.3$) (table 1). There were no significant differences in terms of median time span from illness onset to hospital presentation, or the number of complications [1 (0, 1) versus 1 (1, 1), $p=0.652$]. Overall, 3.9% of patients presented respiratory failure, without a significant difference between those with influenza A (3.9%) and those with influenza B (3.2%), $p=0.571$.

In terms of laboratory tests, fibrinogen was significantly higher in patients with influenza B compared with influenza A ($p=0.005$, $r=0.5$), as were CK ($p=0.071$, $r=0.1$) and CKMB ($p=0.041$, $r=0.2$) (table 1). There were no significant differences in the values of other laboratory parameters such as white blood cell count, neutrophil count, lymphocyte count, other inflammation tests, liver or kidney function.

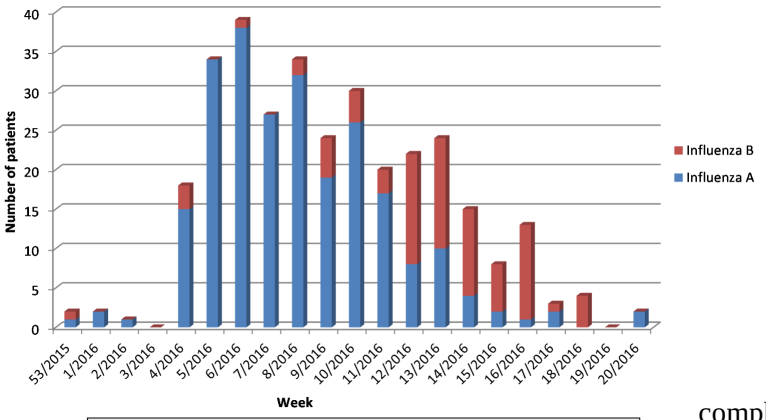

Fig. 1. Seasonal distribution of confirmed cases of influenza in one pediatric ward of a national infectious diseases reference center in Romania

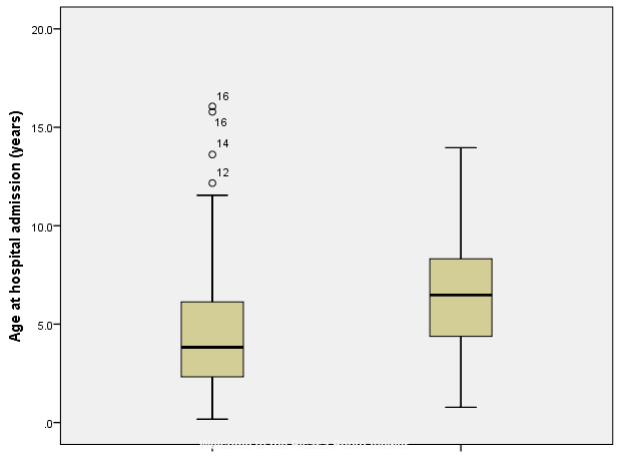

Fig. 2. Age distribution based on type of influenza virus

Table 1

CHARACTERISTICS OF PEDIATRIC PATIENTS WITH INFLUENZA A AND B (STATISTICALLY SIGNIFICANT DIFFERENCES ARE MARKED IN BOLD)

Characteristics, median value (IQR)	Influenza A	Influenza B	Analysis	Effect size
Age at presentation, years	**3.8 (2.3, 6.2)**	**6.5 (4.4, 8.5)**	**p<0.001, U=3490.5**	**r=0.3**
Time to hospital presentation, days	4 (3, 6)	4 (3, 5)	p=0.784, U=4869.5	r=0.0
Duration of hospital admission, days	**5 (4, 6)**	**4 (4, 5)**	**p<0.001, U=3576.51**	**r=0.3**
White blood cells count, cells/μL	6030 (4163, 8688)	5880 (3900, 8390)	p=0.468, U=5202	r=0
Neutrophil count, cells/μL	3200 (1600, 5000)	3.2 (1.7, 5.1)	p=0.700, U=5362.5	r=0
ESR, mm/h	16 (10, 26)	18 (10, 26.5)	p=0.761, U=1762.5	r=0
Fibrinogen, mg/dL	**261 (224, 311)**	**288 (257, 352)**	**p=0.005, U=3128**	**r=0.5**
CRP, mg/L	3.3 (1.3, 9.5)	2.9 (0.9, 12.5)	p=0.920, U=3307.5	r=0
CK, U/L	87 (57, 475)	126 (70, 916)	p=0.071, U=2302.5	r=0.1
CKMB, U/L	**21 (13, 31)**	**24 (16, 37)**	**p=0.041, U=1861.5**	**r=0.2**

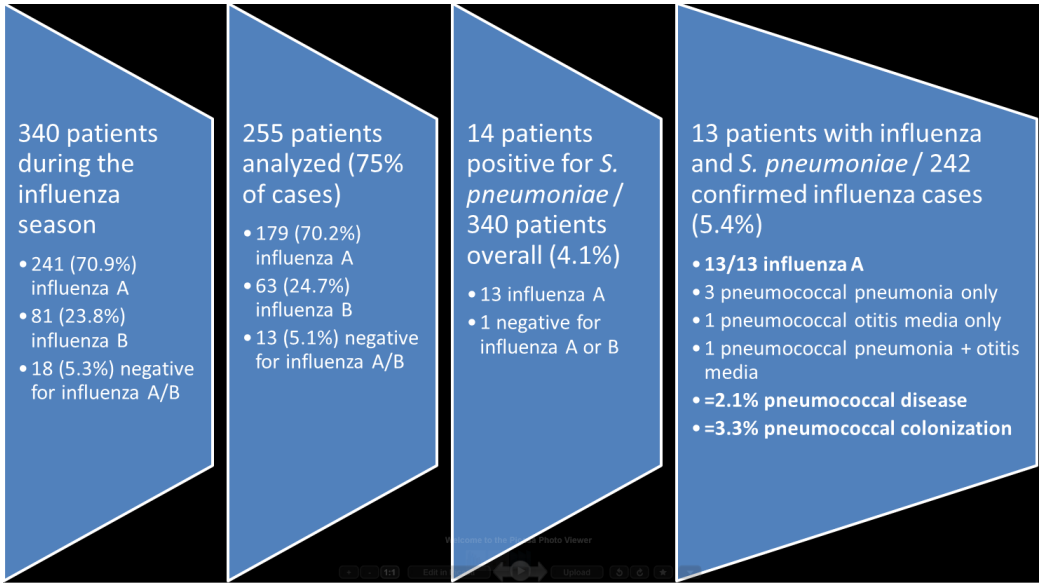

Fig. 3. Study outline and patient distribution

Streptococcus pneumoniae disease or colonization

Out of the total number of 340 patients, 14 were positive for *Streptococcus pneumoniae*. Of these, 13 were cases of influenza A and one case was an influenza-like illness negative for A or B viruses and complicated with serotype 6B pneumococcal bronchopneumonia, in a 5-year-old patient with a past history of pneumonia at 12 months old and repeated upper respiratory tract infections during the past years. For the remainder of the analysis we will refer to the cases of confirmed influenza. The percentage of patients with positive *S. pneumoniae* among those with influenza was 5.4%, all of them being identified in influenza A cases. Four of all cases positive for pneumococcus (an overall 1.7%) presented pneumococcal pneumonia, while two cases presented pneumococcal otitis media-one of these patients developed both otitis and pneumonia, leading to an overall rate of 2.1% pneumococcal disease and 3.3% pneumococcal colonization (fig. 3).

Patients positive for *S. pneumoniae* were slightly younger compared to negative cases ($p=0.164$), presented to the hospital later in the course of disease ($p=0.049$), and required a longer duration of hospital admission ($p=0.016$, table 2), regardless of whether the strains caused disease or were only colonizers (no significant difference, $p=0.524$, $U=15$, $r=0.2$, table 3). Pneumococci were identified after a median (IQR) duration of illness of 5.5 (3.8, 7) days and we identified no significant difference in terms of symptoms or presenting complaint. The risk of pneumococcal positivity was 17.6-fold higher (95%CI: 5.1-61.1) in patients with prior medical history (69.2% vs. 11.4% $p<0.001$, $\div(1)=33.3$).

There were no statistically significant differences in terms of WBC count, but ESR was significantly higher in patients positive for *S. pneumoniae* ($p=0.008$) (table 2). The same trend was present, but the differences were not statistically significant for fibrinogen ($p=0.101$) or CRP ($p=0.063$). However, none of the inflammatory markers presented significant differences between patients with pneumococcal disease compared with colonization (table 3), while neutrophil count was significantly higher in patients with pneumococcal disease ($p=0.011$, $U=3.5$, $r=0.7$). We noted no other significant differences in the values of liver or kidney function tests. Patients with influenza and *S. pneumoniae* were 10.9-fold more likely (95%CI: 2.8-42.0) to present otitis media than those with influenza alone (30.8% vs. 3.9%, $p=0.003$, $\div(1)=17.4$), but we did not find an association between the occurrence of respiratory failure and the presence of pneumococci ($p=0.437$), or that of pneumococcal disease ($p=0.615$).

In all instances pneumococci were identified from nasal swabbing, with the exception of the one case of isolated pneumococcal otitis media and the case of pneumonia plus otitis where nasal and ear swabs were positive. In 12 of the cases pneumococci were recovered in culture, and in one remaining case a multianalyte antigen detection test was positive while cultures remained negative. We

Table 2

CHARACTERISTICS OF PEDIATRIC INFLUENZA PATIENTS WITH OR WITHOUT CONCURRENT IDENTIFICATION OF *S. PNEUMONIAE* (STATISTICALLY SIGNIFICANT DIFFERENCES ARE MARKED IN BOLD)

Characteristics, median value (IQR)	Pneumococcus-positive	Pneumococcus-negative	Analysis	Effect size
Age at presentation, years	3.5 (2.2, 4.7)	4.4 (2.5, 7.0)	p=0.164, U=1147	r=0.1
Time to hospital presentation, days	**5 (3.5, 7)**	**4 (3, 5)**	**p=0.049, U=957**	**r=0.1**
Duration of hospital admission, days	**6 (5, 6.5)**	**5 (4, 6)**	**p=0.016, U=911**	**r=0.2**
White blood cells count, cells/μL	7000 (4030, 8725)	5935 (4090, 8507)	p=0.801, U=1408	r=0
Neutrophil count, cells/μL	2800 (2060, 5490)	3250 (1600, 5060)	p=0.869, U=1429	r=0
ESR, mm/h	**26 (22, 37)**	**15 (10, 25)**	**p=0.008, U=223**	**r=0.2**
Fibrinogen, mg/dL	307 (249, 358)	269 (233, 320)	p=0.101, U=933	r=0.1
CRP, mg/L	7.0 (3.6, 11.1)	2.9 (1.1, 9.6)	p=0.063, U=858.5	r=0.1

identified an overall rate of 12.5% resistance and 62.5% intermediate susceptibility to penicillin, 81.8% resistance to erythromycin, 72.7% resistance to clindamycin, uniform resistance to co-trimoxazole, and no resistance to vancomycin (table 3). Serotyping was performed for 9 of the strains, identifying the following serotypes: 19 (n=4), 6 (n=2), 9, 11 and 14 (n=1 each). Of the five cases of pneumococcal disease, serotyping information is only available for two of the strains (table 3). One was a serotype 9 *S. pneumoniae* involved in otitis media that preceded influenza illness by two weeks, in an 18-months old boy with personal medical history of atopic dermatitis and cow's milk protein allergy, and the second a serotype 19 strain responsible for acute pneumococcal pneumonia in a 30-months old boy with negative personal medical history. Serotypes 6, 11, and 14 were all involved in colonization and not in pneumococcal disease. Two of the children had been vaccinated with pneumococcal-conjugate vaccine 13 (PCV13) active against the following serotypes: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In both cases pneumococci did not cause disease and were only involved in asymptomatic nasal colonization; serotypes 11 and 19 were identified, respectively.

Our study has identified a low rate of pneumococcal colonization (3.3%) and an even lower rate of pneumococcal disease (2.1%) in pediatric patients with influenza, but unfortunately complete data regarding vaccination against *S. pneumoniae* was not available in this study. These data are surprising given the well described mechanisms and interactions between influenza virus and pneumococci: influenza virus has been shown block mucociliary clearance and lead to increased bacterial [pneumococcal[3] and staphylococcal [7,8] densities while also inducing the production of interferon â by lung macrophages infected by influenza virus, and this associates a dramatic reduction in bacterial phagocytosis, specifically for pneumococci.[4] These findings are encouraging, as coinfection with influenza A virus and *S. pneumoniae* may also be responsible for altered pharmacokinetic and pharmacodynamic responses to oseltamivir, with a decrease in antiviral efficacy to 47%[9].

In our study penicillin-non-susceptibility was lower compared to rates ranging from 46.7%[10] to 94%[11] reported in pathogenic pneumococci in Romania, but higher compared with rates of 14% in Brazil[12] and India[13], or 37.8% in the USA[14]. Moreover, we identified high rates of non-susceptibility to antimicrobials such as erythromycin (81.8%), clindamycin (72.7%) and co-trimoxazole (100%), somewhat comparable to rates of 97.7%, 89.95% and 51.2% in China[15], while the national Romanian estimate for 2014 was 48% non-susceptibility to macrolides[10], almost twice as low as the figures reported in our study. These rates are worrisome, but may not display an accurate picture of pneumococcal resistance, as the number of cultured strains was low (n=12). However, this suggests the need to readapt the existing algorithms for assessing the risk of antimicrobial resistance, as the available tools are mostly applicable to Gram-negative bacilli rather than Gram-positive cocci[8,16].

The rate of pneumococcal positivity in our study was 17.6-fold higher in patients with prior medical history ($p<0.001$), and the presence of pneumococci increased the duration of hospital admission ($p=0.016$), regardless of whether they generated disease or were only colonizers. When pneumococci were present, the risk of otitis media was increased 10.9-fold ($p=0.003$).

Table 3

CHARACTERISTICS OF PEDIATRIC PATIENTS WITH INFLUENZA A AND PNEUMOCOCCAL DISEASE OR COLONIZATION (STATISTICALLY SIGNIFICANT DIFFERENCES ARE MARKED IN BOLD)

Characteristics, median value (IQR)	Pneumococcal disease	Pneumococcal colonization	Analysis	Effect size
Age at presentation, years	3.5 (2.1, 4.1)	3.6 (2.0, 4.8)	p=0.622, U=16	r=0.2
Time to hospital presentation, days	7 (4, 7)	5 (3.3, 5.8)	p=0.284, U=12.5	r=0.3
Duration of hospital admission, days	6 (5.5, 9.5)	5.5 (5, 6.8)	p=0.524, U=15	r=0.2
White blood cells count, cells/μL	7790 (7200, 8725)	4850 (3543, 8328)	p=0.171, U=10	r=0.4
Neutrophil count, cells/μL	**4880 (4150, 6135)**	**2310 (1418, 2790)**	**p=0.011, U=3.5**	**r=0.7**
ESR, mm/h	26 (26, 35)	27 (15.5, 37)	p=0.686, U=6.5	r=0.2
Fibrinogen, mg/dL	311 (281, 431)	292 (242, 330)	p=0.222, U=11	r=0.4
CRP, mg/L*	12.3±7.9	5.9±3.8	p=0.072, t(11)=1.987	d=1.0
Penicillin-non-susceptible strains**	1 (20%)	0 (0%)	p=0.093, z-score=1.3	N/A
Penicillin-reduced-susceptibility strains**	**0 (0%)**	**4 (50%)**	**p=0.029, z-score=-1.9**	**N/A**
Erythromycin-non-susceptible strains**	3 (60%)	5 (62.5%)	p=0.464, z-score=-0.1	N/A
Clindamycin-non-susceptible strains**	2 (40%)	5 (62.5%)	p=0.215, z-score=-0.8	N/A
Co-trimoxazole-non-susceptible strains**	3 (60%)	7 (87.5)	p=0.127, z-score=-1.2	N/A
Vancomycin-non-susceptible strains**	0 (0%)	0 (0%)	N/A	N/A
S. pneumoniae serotypes	9, 19, three missing serotypes	6, 6, 11, 14, 19, 19, 19, one missing serotype	N/A	N/A

*Mean and standard deviation are presented for variables with normal distribution, along with the results of the independent samples t test and its corresponding effect size. **Number of strains and percentage in each category are reported, along with the results of the z test for proportions. For pneumococcal disease N=5, for pneumococcal colonization N=8.

We identified pneumococci only in patients with influenza A infection and not in those with influenza B infection, but this finding is not surprising, as there are relatively few reports of pneumococcal infection following influenza B[2,17], probably due to the fairly scarce interactions seen between pneumococci and influenza B virus, as shown by the virtually unaltered pro-inflammatory cytokine profile expressed by bronchial epithelial cells exposed to these germs[18].

Pneumococci are known to induce a strong inflammatory response in the host, which is accountable for the pathogenesis of pneumococcal disease. However, recent studies have shown that an inflammatory response may also be present in asymptomatic nasopharyngeal colonization[19], but to date there are no specific studies in field literature describing the exact role of each of the commonly used inflammation biomarkers in detecting or differentiating between pneumococcal colonization. Our study is therefore the first assessment of the utility of widely available laboratory tests in pneumococcal colonization/ infection. We have shown that ESR is the only inflammatory marker that appears to be a good indicator of pneumococcal presence ($p=0.008$) but does not distinguish between disease and colonization ($p=0.857$).

We have shown that ESR appears to be a good indicator of pneumococcal presence ($p=0.008$, $r=0.2$) but does not distinguish between disease and colonization ($p=0.686$), as opposed to the neutrophil count, whose higher values were strongly associated with disease rather than colonization ($p=0.011$, $r=0.7$).

Conclusions

In the 2015-2016 pediatric influenza season, influenza A occurred in patients who were significantly younger and required longer hospitalization compared to those with influenza B. Pneumococcal colonization (3.3% of cases) or disease (2.1% of cases) occurred exclusively in patients with influenza A, specifically in patients who were younger, presented to the hospital later and had higher ESR values compared to negative cases. Commonly used markers of inflammation may be used to identify the presence of pneumococci (ESR) or to differentiate between colonization and disease (neutrophil count) in children with influenza A.

In conclusion, commonly used markers of inflammation may be used to identify the presence of pneumococci (ESR) or to differentiate between colonization and disease (neutrophil count) in children with influenza A.

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Abbreviations

CRP- C-reactive protein;
ESR- erythrocyte sedimentation rate;
IQR - interquartile range;
WBC- white blood cells count.

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