

CD64 expression on monocytes in children with adenoid hypertrophy

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Summary

Objective: Adenoids with obstructive hypertrophy (AH) are considered to be one of the most ancient and common problems in Paediatrics. This study aimed at investigating the expression of CD 64 on monocytes in children who had to undergo adenoidectomy.

Methods: Therefore, 66 children (24 females, 42 males, mean age 4.9 years), affected by AH and requiring adenoidectomy (54%) or adenotonsillectomy (46%) were consecutively enrolled into the study. Moreover, 30 healthy children (11 females, 19 males, mean age 11.2 years) were enrolled in this study as controls. CD64 expression on monocytes was measured.

Results: Children with severe AH had higher CD64 values than children with AH size 3 and controls.

Conclusions: This preliminary study shows that CD64 expression on monocytes may be associated with severe AH, suggesting a more intense immune activation in these subjects. (*Asian Pac J Allergy Immunol* 2013;31:132-7)

Key words: Adenoid Hypertrophy, CD64, monocytes

Introduction

Studies on the usability of monocyte FcγRI in clinical infections diagnostics stimulated an increased research interest.¹ Indeed, several papers have been published about the expression of FcγRI on the surface of monocytes.²⁻⁵

Human cells of myeloid lineage contain 3 classes of receptors for the Fc portion of immunoglobulin G, designated FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16), which act in concert with complement receptors to provide an essential link between the humoral and cellular immune systems by functioning as key molecules in antigen presentation, phagocytosis, clearance of immune complexes, and release of inflammatory mediators.⁶ FcγRI binds monomeric human IgG1 and IgG3 with high affinity, so leading to saturation, but binds C-reactive protein with low affinity.⁷

Human FcγRI is constitutively expressed on professional antigen-presenting cells (such as monocytes, macrophages, and dendritic cells) which express on their surface major histocompatibility complex class II molecules.⁸ Membrane-bound FcγRI on antigen-presenting cells has been found to be fundamental in mediating enhanced antigen presentation through internalization of antigen forming complexes with IgG.⁹ Thus, as antigen presentation is essential for stimulating an effective immune response, the measurement of CD64 on monocytes may be useful in patients with infectious disorders. Therefore, CD64 might be considered a general marker of inflammation/infection.¹

Adenoids with obstructive hypertrophy are considered to be one of the most ancient and common problems in Paediatrics. Anatomically, adenoids are part of Waldeyer's ring and, since they may create mechanical Eustachian tube (ET) obstruction, they are relevant in the pathogenesis of otitis media (OM). In addition, to diagnose adenoid hypertrophy (AH), nowadays nasal endoscopy is considered to be the gold standard even in young kids, as this technique is also able to detect a possible association between adenoid inflammation/infection and OM, especially during infancy and

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early childhood.¹⁰ Adenoids removed for airway obstruction and/or recurrent infections have been studied to identify a possible mechanism to explain chronicity, and studies have shown the presence of bacteria organized into biofilms which provides germs with a greater capability to resist against host immunological defenses and antibiotics, and may therefore promote the recurrence or the persistence of infections.¹¹ Biofilm formation is detectable also on adenoid surfaces, particularly in children with recurrent infections.¹² In addition, immune competent cells, including macrophages/monocytes, are recruited and activated in children with recurrent infections.¹³ In this setting, a vicious circle occurs: activated cells release factors that allow chronic inflammation/infection. In this regard, monocytes may play a relevant pathogenic role in those children with AH and recurrent infections. However, there is no data concerning the precise role of CD64 expression on monocytes in children with adenoidal problems. Therefore, this study was aimed at investigating CD64 expression on monocytes in children who had to undergo to adenoidectomy because of adenoid hypertrophy (AH).

Methods

Patients

In all, 66 children (24 females, 42 males, mean age 4.9 years), affected by persistent upper airway obstruction caused by adenoid hypertrophy and requiring adenoidectomy (54%) or adenotonsillectomy (46%) were consecutively enrolled into the study. Moreover, 30 healthy children (11 females, 19 males, mean age 11.2 years) were enrolled in this study as controls. Patients were recruited between February and October 2011 at the Department of Otorhinolaryngology at the Fondazione IRCCS Policlinico San Matteo Pavia. Control subjects were healthy and parents reported that their children did not suffer from any inflammatory/autoimmune disorders and/or cardiovascular, pulmonary or metabolic diseases, and were symptomless (and consequently not taking any drug treatments).

The study was approved by the Institutional Review Board and written informed consent was obtained from the parents of the children.

Clinical evaluation was measured, considering these parameters: i) frequencies and severity of infection (URI, Upper Respiratory Inflammation; LRTI, Lower Respiratory Tract Inflammation); ii) snore; and iii) sleep apnoea.

The patients were evaluated by nasal endoscopy for adenoid hypertrophy. The adenoids were graded

in according to Parikh's classification¹⁴: a score of +1 indicated an adenoid size of 0 to 25% of the retro-palatal airway; a score of +2 indicated an adenoid size of 25% to 50% of the retro-palatal airway; a score of 3+ indicated an adenoid size of 50 to 75% of the retro-palatal airway; and a score of 4+ indicated an adenoid size >75% of the retro-palatal airway lumen.

Tonsil size was classified according to validated criteria¹⁵ as follows: grade 0: no obstruction in airway; grade 1: < 25% obstruction in airway; grade 2: 25-50% obstruction in airway; grade 3: 50-75% obstruction in airway; grade 4: > 75% obstruction in airway.

Primary snoring (PS) and obstructive sleep apnea syndrome (OSAS) could overlap in some children, and only polysomnography (PSG) can differentiate between the two disorders. However, there is a long-standing debate over the need to carry out such a complex and expensive test in all children that present symptoms suggestive either for PS or OSAS.¹⁶ As reported also by some authors,¹⁷ our approach to sleep disordered-breathing (SDB) in children is primarily based on clinical assessment, while PSG is reserved for selected cases. Patient evaluation included taking a clinical history with the help of parents. The suspicion of "OSAS" was suggested by the presence of habitual snoring (constant nocturnal snoring in the absence of concomitant respiratory infection) for at least 6 months; frequent episodes of apnea (interrupted breathing for more than 10 seconds, followed by a sudden noisy breath); fragmented sleep (frequent changes of position or awakening). Those children who did not manifest these features were classified as "PS".

Sample collection and detection

Blood samples from patients were obtained at admission to the hospital, before adenoidectomy. Samples were collected from patients and controls to determine monocyte FcγRI (CD64) from whole blood.

Monocyte FcγRI CD64 analysis was performed according to a standardized procedure described by Bakke et al.¹⁸ Fifty μl of whole blood were stained with a combination of anti-CD64-PE (provided by Becton-Dickinson, San Jose, CA) for 60 min in the dark followed by red blood cell lysis, no washing, and an additional 60-min incubation. The specimens were analyzed on a FACSCalibur flow cytometer (Becton-Dickinson) with an FL3 threshold. QuantiBRITE PE beads (Becton Dickinson) contain a mixture of four different beads with a known

number of bound PE molecules. A standard curve of regression comparing the mean fluorescence intensity of the stained cells to the number of PE molecules can be created with Microsoft Excel software allowing the determination of the antibody binding capacity (ABC) of CD64. In fact, since there is one PE fluorescent molecule per antibody it is possible to predict the number of CD64 molecules present on the stained cells following the evaluation of CD64 PE mean fluorescence intensity. Although this ABC may not be identical to the CD64 expression, it is related to the actual CD64 expression; for the rest of this discussion, CD64 expression and antibody binding capacity will be considered the same. No isotype control was used. The anti-CD64 reagent was titrated to saturation (Becton- Dickinson). Non-specific staining was reduced by adding a double volume of red blood cell lysing reagent (FACS Lyse, Becton-Dickinson) and incubating for an additional 60 min before data acquisition. The CV of repeat measurements was 3–6%.

Statistical analysis

Descriptive statistics were first performed and quantitative parameters were reported as medians (md) with first and third quartiles (IQR) due to the skewed distribution. Qualitative data were reported as frequencies and percentages. Association of qualitative data among various groups of patients was made by the chi-squared test. Comparison of quantitative variables was made by the non-parametric Wilcoxon test. Correlation between quantitative variables was evaluated by means of the Spearman's correlation coefficient (ρ). All tests were two sided and a p -value less than 0.05 was considered statistically significant. The software Medcalc 9 (Frank Schoonjans, BE) was used for all analyses.

Results

Baseline characteristics of patients

The demographics and clinical characteristics of patients and healthy children are shown in Table 1. The frequencies of monocytes and granulocytes are reported in Table 2: there was a significant difference in the expression of CD64+ on monocytes (p -value = 0.0355) between patients and controls, as shown in Figure 1. Recurrent upper respiratory tract infections were present in 17

Table 1. Demographics and clinical characteristics of patients and healthy children

	Patients	Healthy children
Number	66 children	30 children
Males (nr; %)	42 males; 63.6%	19 males; 63.3%
Age (median; range)	5 years; 2-10 years	10 years; 8-12 years
Presence of URI (nr. of patients)	17	0
Presence of LRTI (nr. of patients)	10	0
Presence of snore (nr. of patients)	62	2
Presence of sleep apnoea (nr. of patients)	44	1
Adenoid size (nr. of patients)	3 (28 pt) 4 (38 pt)	1 (28 pt) 2 (2 pt)

children, and 10 children had RLRTI. Snoring was very frequent, occurring in 62 children, as was sleep apnoea (44 children). With regard to adenoid size, 28 children had size 3 and 38 size 4. The patients were divided in two groups, the first with an adenoid size of 3, and the second with an adenoid size of 4. There was a significant difference between the two groups ($p = 0.003$) as reported in Figure 2. Children with larger adenoid size showed higher CD64 expression values. In addition, only the size 4 group had higher CD64 levels than those in the control group ($p = 0.0002$).

There was no association between adenoid size score and clinical parameters, including RURTI (p -value = 0.5435), RLRTI (p -value = 0.7013), and sleep apnoea (p -value = 0.2053).

Table 2. Distribution of CD64 (% and number of sites) and monocytes on cell CD64+ (%), evaluating patients and healthy children separately. Data were expressed as median (md) and interquartile ranges (IQR), and p -values between groups (Wilcoxon test).

	Patients	Healthy children	p -value
CD64 (nr of sites)	md: 2376 (IQR: 2171-2456)	md: 2369 (IQR: 2200.3-2391)	0.2876
CD64 (%)	md: 13.5% (IQR: 8.9-16.1%)	md: 10.2% (IQR: 7.9-17.9%)	0.4259
% Monocytes on cells CD64+	md: 5.1% (IQR: 4.3-6.6%)	md: 6.6% (IQR: 5.1-8%)	0.0355

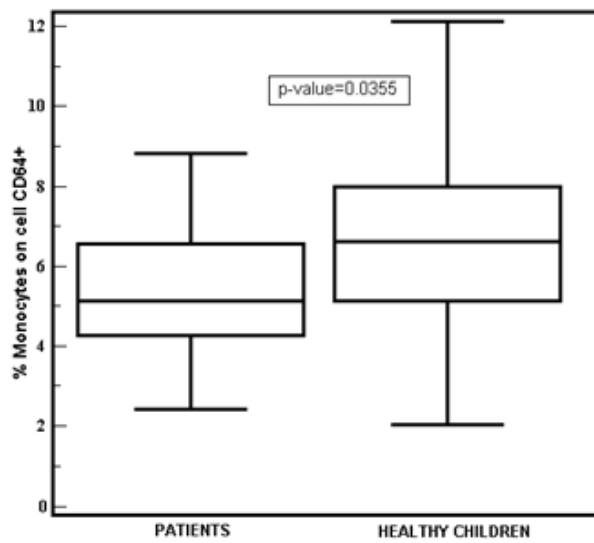


Figure 1. Distribution of monocytes on cell CD64+ (%), in patients and healthy children. Values were represented as median (black line), quartiles (25th-75th percentiles, boxes) and p-value between groups

Discussion

Adenoid hypertrophy represents a relevant problem for both the paediatrician and the ENT specialist. AH, which obstructs the nasal airway in children, is associated with numerous symptoms, including: snorting, snoring, nasal obstruction, oral breathing, rhinolalia, restless sleep, hypersomnolence, and enuresis.¹⁹⁻²¹ AH is also the most common cause of obstructive sleep apnoea and cardio-respiratory syndrome, with severe complications.^{20,22} Moreover, AH plays a major role in paediatric chronic rhinosinusitis and chronic otitis media. In addition, children with adenoid hypertrophy may quite frequently have recurrence of respiratory infection both at upper and lower level.²²

On the other hand, there is no study that has investigated the possible role of some protein as a surrogate marker of AH in children. Only one study investigated the CD64 expression on leukocytes in adult patients suffering from obstructive sleep apnea (OSA).²³ This study reported that CD64 expression on monocytes was reduced in OSA patients in comparison with control subjects. However, this study was conducted in patients with a mean age of 49.3 (+11.0) years and adenoid size was not considered.

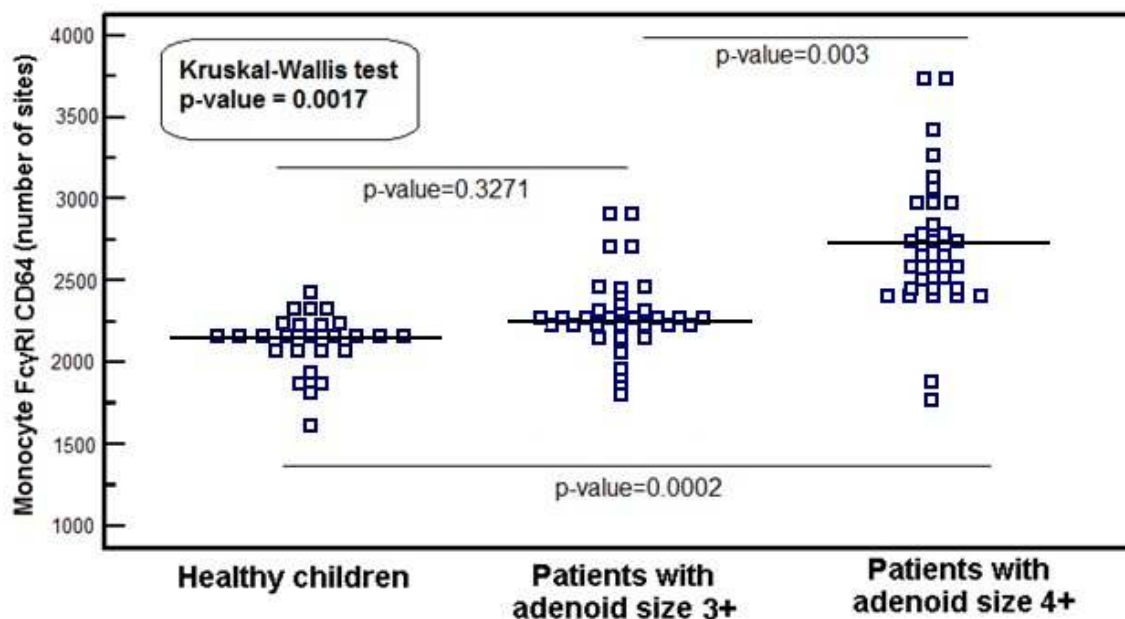


Figure 2. Distribution of monocyte Fc γ RI CD64 levels, expressed as number of sites, in patients with adenoid size of 3+ and patients with adenoid size of 4+. Single values were represented together with medians (solid black line).

Adenoids may be frequently the site of chronic inflammation and many inflammatory cells may contribute to this phenomenon. Monocytes/macrophages, but also neutrophils, and eosinophils seem to play a pivotal role in this issue. Therefore, this study aimed at investigating the possible role of CD64 expression on monocytes in this regard.

The present study reported some interesting findings. Firstly, a relevant percentage of children with AH had recurrent respiratory infections, mainly at upper level, almost all of them snored and a significant proportion had sleep apnoea; half had adenoid size 3 and half adenoid size 4.

Secondly, children with size 4 AH had the highest CD64 expression levels. This significant association could be related to the recurrence of airway infections that stimulate monocytes. Therefore, a vicious circle might start involving adenoid tissue enlargement, immune system stimulation, and airway infections. Therefore, CD64 assessment might be useful in children with AH. Of course, further studies should be conducted enrolling a larger sample of AH children and considering an adequate follow-up to confirm these preliminary findings.

Furthermore, two recent studies provided evidence that leukotriene receptor antagonists may be a therapeutic alternative in children with sleep-disordered breathing avoiding adenoidectomy.^{24,25} The positive effect of antileukotrienes could be due to their anti-inflammatory activity. Therefore, these studies could support the thesis that there is an immune-mediated inflammation in children with AH.

On the other hand, it is of note that this study has a relevant limitation as the age of patients and control group are not well matched and this constitutes a methodological bias as immune parameters change with age. In addition, study of CD64 expression on monocytes from adenoid tissue may be needed to confirm the association.

In conclusion, this preliminary study shows that CD64 expression on monocytes is associated with severe AH, meaning a more intense immune activation in these subjects.

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