

Chronic inflammatory diseases in children are more severe in girls

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Abstract

In humans and animal models, females express higher immune reactivity and more robust inflammatory responses. We analysed the expression of current inflammatory markers in 149 children (74 females / 75 males) suffering from three chronic inflammatory diseases: 50 asthma, 47 cystic fibrosis and 52 sickle cell anaemia to evaluate potential differences in clinical response according to gender. Data including temperature, neutrophil count (NC) and C-reactive protein (CRP) were recorded for each patient at several time points according their disease.

In asthma, NC was higher in females than in males ($p < 0.02$), as were doses of cortisone ($p < 0.04$) or inhaled bronchodilators ($p < 0.01$) received at recovery.

In cystic fibrosis, NC became significantly higher in females at age 5 ($p < 0.003$), while episodes of infection and antibiotic administration were already significantly more frequent in females at age 2 ($p < 0.02$ and $p < 0.05$ respectively).

In sickle cell anemia, the number of crises since diagnosis and number of acute chest syndrome (ACS) episodes were significantly higher in females ($p < 0.01$ and $p < 0.05$ respectively). Our study extends the documentation of a relationship between gender, inflammatory markers and clinical outcome in pre-pubescent children, suggesting a genetic predetermination is more likely than hormonal influence.

Key Words: inflammation, gender differences, acute asthma, cystic fibrosis, sickle cell anaemia, CRP, ESR, neutrophil count.

Introduction

Clinical reports and experimental data support the hypothesis that gender can affect the prevalence, severity and natural history of inflammatory conditions (1-5).

In humans and animal models, females express higher immune reactivity than males with increased resistance to infections, but also higher susceptibility to more deleterious autoimmune diseases and to exaggerated inflammatory processes (asthma, cystic fibrosis) (6-10). Women demonstrate poorer asthma control, despite reporting higher rates of inhalation corticosteroid use and routine asthma care visits (11, 12). More women than men go to emergency departments due to asthma symptoms, and are admitted to the hospital. Similarly, in chronic obstructive pulmonary disease (COPD), the number of female deaths now surpasses that for men in the United States. As COPD is a disease of inflammation, gender variations in the inflammatory response could therefore be responsible for gender differences in the disease (13).

Young women with metabolic syndrome show increased stiffness of peripheral and central arteries, a mechanism that can be associated to higher inflammation and might explain their increased cardiovascular risk i.e. they have poorer outcomes from stroke than men (14).

In 1997, Rosenfeld *et al* (15) conducted a large study in individuals aged 1-20 years, suffering from cystic fibrosis (CF) treated in the US Centers, and found that females had poorer survival than males. This "gender gap" was not explained by a wide variety of potential risk factors. In CF, inflammation is already present before birth and is thus a life long sustained condition and could determine the poor prognosis of females. Although during childhood and adolescence, lung

function and nutritional status are usually equivalent in patients of both genders, owing to quality of care (16).

In fact, inflammation is a double edge sword: when patients are in good health inflammation remains a very efficient process to avoid important exogenous aggression, systemic life threatening or major local infections. However, when inflammation persists, collateral deleterious effects of tissue destruction outweigh the initial advantage.

Animal model (18) and human observations (19) suggest that a more robust inflammatory response occurs in females than in males. Considering people of far-advanced age, demographic data document a clear-cut prevalence of females compared to males, suggesting that gender-specific mortality rates follow different trajectories during aging. More effective immune reactions against infectious diseases may also give advantages to females.

In contrast, few clinical data on gender influences are available from pre-pubescent patients. Considering the surgical stress of cardiac operations, females recover better than males (20), suggesting a more efficient inflammatory (and perhaps secondary anti-inflammatory) response facing a similar level of external insult, limited in time and extent (surgical trauma and extra corporeal circulation). In more complex situations such as CF and autoimmunity, females have a poorer prognosis (15). Persistent inflammation could involve both a lower control of underlying cause of inflammation (infection for ex.) and consequent collateral tissue damage resulting from extended mobilisation of inflammatory cells, regardless of excellent anti-inflammatory reaction-like production of anti-proteases (also members of acute phase proteins) .

Females seem to be protected against deleterious effects of cardiac operations, suggesting a better recovery from their inflammatory status after surgery, a relative acute situation (20). In contrast, adult females have a poorer prognosis in cystic fibrosis (15) and many autoimmune diseases, a chronic situation.

To evaluate gender differences before puberty in various paediatric conditions characterised by chronic inflammation, we analysed the expression of current inflammatory markers (C-reactive protein, erythrocyte sedimentation rate and neutrophil count) and recorded clinical data in children with underlying inflammation in three chronic inflammatory diseases (asthma, cystic fibrosis and inflammation produced by recurrent crisis in sickle cell anaemia) As asthma and cystic fibrosis, inflammatory processes are very aggressive in sickle cell anaemia when severe crisis is observed (21).

Subjects and methods

The study included 149 children (74 girls and 75 boys) hospitalised at the University Children's Hospital Queen Fabiola between January 1999 and March 2001: This population included 50 children hospitalised for acute exacerbations of chronic asthma treated by corticosteroids, (25 girls and 25 boys the first 25 consecutive females were matched to the same number of males), 52 children with cystic fibrosis (26 girls and 26 boys out of a cohort of 75 children) followed in outpatient clinic and evaluated for this study successively at 2 and 5 years of age and 47 children with severe sickle cell anaemia (23 girls and 24 boys successively enrolled) regularly hospitalised for vaso-occlusive crises (VOC).

In the three groups, patients were matched for age and sex and then recorded for study purposes according to the date of admittance or to the date of scheduled visit in group 2. All patients were less than 8 years old in cases of asthma and cystic fibrosis, and between 23 and 163 months in cases of sickle cell anaemia (mean age of 7.5 months, not different for girls and boys).

The study was approved by the Ethical Committee of the University Children's Hospital Queen Fabiola.

Acute asthma crisis (group 1)

Patients were enrolled in this group on the basis of presence of severe acute bronchospasm not reversible by inhaled salbutamol at a dose of 200 microgrammes. For each patient, temperature at admittance, white blood cell count (WBC) and formula, C-reactive protein (CRP) were collected at admittance as well as the number of days of aerosols (inhaled bronchodilators) and days of hospitalisation. Cumulative doses of cortisone, theophyllin, salbutamol and ipratropium bromide were recorded until the auscultation returned to normal.

Cystic fibrosis (group 2)

For each patient, data were retrospectively collected at 2 and 5 years of age in steady state avoiding acute infectious conditions. WBC count and NC with formula, erythrocyte sedimentation rate (ESR), days of antibiotic administration during the last 12 months and total number of infectious episodes (defined by the need of antibiotic therapy both intravenous and/or enteric) were recorded. One second forced expiratory flow (FEV 1) was measured and patient height was measured at 6 years of age. One positive culture by *Pseudomonas aeruginosae* was considered in both girls and boys as a major risk factor.

Sickle cell anaemia (group 3)

For each patient data were collected on age of first crisis, total number of crises since diagnosis, total number of days hospitalisation for VOC, total number of Acute Chest Syndrome (ACS) episodes, frequency of treatment by Hydroxyurea (HU) as well as WBC, NC, CRP and ESR measurements at last control in steady state.

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)

Data and blood were collected from routine screening tests starting at admission (day care for CF or current hospitalisation in case of asthma exacerbation).

CRP was measured by turbidimetric immunoassay using the kit from APTEC Diagnostic® Hamme, Belgium, calibrated according to IFCC recommendations (ref. CRM 470). ESR was

estimated over one hour using the erythrocyte sedimentation rate reader from Menarini, Menarini Diagnostic (Paris, France).

Neutrophil counts

Neutrophil counts were estimated using the reader from Siemens Healthcare Diagnostic, (Brussels, Belgium).

Statistical Analysis

A Student t-test with 95% confidence limits was used to compare means, Chi² Test for cross tabulations and the Mann-Whitney test was used for nonparametric variables. Related variables were determined by multiple logistic regression analysis and relationships between multiple nonparametric groups of variables were determined by the Kruskal-Wallis test.

Results

Acute exacerbations in chronic asthma

Results are presented in table 1.

Body temperature (normal limits) at admittance was not different in girls and boys (mean $\pm$ SD in girls = 36.5°C $\pm$ 0.4 °C; mean + SD in boys = 36.7°C $\pm$ 0.4 °C)

Neutrophil count was higher in girls than in boys ($p < 0.03$). C-reactive protein concentrations were not significantly different ($p < 0.16$) even if higher in girls. At clinical recovery, females had received significantly more intravenous corticosteroids (median cumulative dose since admission) ($p < 0.03$) and nebulization days of inhaled bronchodilators was also significantly higher in females than in males ($p < 0.01$). Cumulative doses of salbutamol, ipratropium bromide and theophyllin were higher in girls than in boys but were not statistically significance. The number of days of hospitalisation was higher in girls ($p < 0.03$ one side).

Cystic fibrosis

Results are summarised in table 2.

WBC and NC were not different at 2 years of age but became significantly higher in girls compared to boys at age 5 (for WBC $p < 0.043$); (for N at 5 years $p < 0.043$). Erythrocyte sedimentation rates were not different in either gender at 2 and 5 years of age, but were higher in girls than in boys. However, the total number of episodes of infection and days of intravenous antibiotics were significantly higher in females from 2 years of age: (median total number of infectious episodes, 25-75 percentile in girls = 6 (4-7); median, 25-75 percentile in boys = 3.5 (2-4) ($p < 0.02$).

Only girls were receiving intravenous antibiotics at 2 years of age ($p < 0.05$).

Expected FEV 1 at age 5 and mean weight percentile were not different in either gender.

Sickle cell anaemia

Results are summarised in table 3.

The median age at first VOC was lower in girls than in boys but not significantly different.

The total number of crises since diagnosis and number of acute chest syndrome episodes were significantly higher in females than in males, girls experiencing almost 2 times more VOC and ACS. Number of crises since diagnosis ($r = 0.48$ $p < 0.02$) and days of hospitalisation ($r = 0.42$ $p < 0.05$) were significantly correlated with age in girls but not in boys.

Discussion

This study, in pre-pubescent children, describes a relationship between gender, clinical outcome and inflammatory markers (CRP, neutrophil count). Possible differences in immune reactivity due to age associated maturation were excluded since male and female subjects were age-matched. Gender differences in clinical outcome indicate that chronic inflammation events, underlying the physiological mechanism of disease, could be more damaging in females in very

early life, than in males. Enhanced levels of inflammation in females and potentially higher recruitment of neutrophils as activated cells of the disease could be deleterious due to chronic tissue damage in various organs (lung, liver, intestine, spleen). It is at the present time difficult to establish whether the more severe condition observed in females is dependant from a continuous process or if it is the result of repeated acute exacerbations. This is also true independently of the gender difference when considering the progressive alteration of lung function in CF patients: we are not able to distinguish between the effects of continuous versus repeated tissue damages, but the clinical result consists in patient degradation.

However our observations could extend the documentation of previous data showing a gender gap in cystic fibrosis mortality (15) in an American cohort (more than 21000 patients aged 1 and 20 years), where girls had a 60% higher risk than boys. This difference was not accounted for by the examination of risk factors, including nutritional status, pulmonary function and pathogen in sputum, pancreas insufficiency or age at diagnosis. This is also verified in cases of severe asthma (22) that are more frequent in females and more persistent in adulthood, with higher mortality after 40 years of age. Recent data from a North American population also showed that males were 46% less likely to have an asthma exacerbation than females (OR 0.54, 95% CI: 0.31-0.94) (23). Our data in pre-pubescent children hospitalised for severe exacerbations of chronic asthma, support the hypothesis that inflammation is enhanced in girls, requiring higher doses of cortisone, longer duration of inhaled therapy and finally longer stays in hospital. In chronic bronchial inflammation (cystic fibrosis and severe asthma), our data raise the question of preventive and/or chronic anti-inflammatory treatment, which could be adapted quantitatively and qualitatively according to gender, to prevent greater damage in females compared to males. If these data were confirmed in larger prospective studies, prevention strategies in cystic fibrosis could consider early introduction of systemic or inhaled anti- inflammatory drugs, provided that side effects

were minimised, especially in females. In a recent study, others showed an unexplained worse prognosis in female suffering from sickle cell anemia according to lower forced vital capacity. (24)

Prior to this study, no significant physiological differences in inflammatory response between very young girls and boys had been reported in several chronic inflammatory diseases. Gender differences in adulthood inflammatory responses can be related to sex hormone production. In pre-pubescent children, levels of sex hormones are very low, although mean levels of sex hormones are still 7 times higher in females than in males (25). Whether these hormonal differences at a very low level could modulate differences in inflammatory response patterns between genders is unclear. We totally agree with the assertion of Fisch (26) declaring that despite accumulating evidence supporting sex-based differences in innate and adaptive immune responses, in the susceptibility to infection or in the prevalence of autoimmune diseases, research and clinical practice do not address these distinctions, and most studies of immune responses do not stratify by sex. X-linked genes and hormones are among the many factors that could contribute to disparate immune responses in males and females. In the future, it is crucial to address sex-based differences in disease pathogenesis and in the pharmacokinetics and pharmacodynamics of therapeutic medications to provide optimal disease management for both sexes.

Our observations could suggest that direct genetic influences due to differential expression of several genes associated with X chromosome may be involved. Indeed, the partial inactivation of the X chromosome in females and differences in the expression of certain genes have been reviewed by N Ellis (27) and, more recently discussed by Z Spolarics (28). Interestingly, some of these genes are involved in the complex interactions of adhesion molecules, cytokines, their receptors, and signal transduction. This gender variation could interfere with aspects of cell

function, resulting in different responses in acute versus sustained inflammation. Neutrophils appear to play a central role in mobilisation and production of cytokines, with earlier and more intense recruitment in females.

We recently published a paper (29) demonstrating clear gender differences in inflammatory marker expression during current acute infectious diseases.

In fact, inflammation is a double edge sword. When patients are in good health, inflammation remains a very efficient process to avoid important exogenous aggression of systemic life threatening (as major thermal burns) or major local infections (30-33). In contrast, when inflammation persists, collateral deleterious effects of tissue destruction outweigh the initial advantage.

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Table 1: Outcome after acute crisis of severe asthmatic patients according to gender

	females medians (p25-75)	males medians (p25-75)	MW (P value)
cortisone mg (cumulative dose since admission)	500 (268-908)	250 (183-333)	<0.03
Nr of days aerosol	2.5 (1-3.6)	1 (1-1.3)	<0.04 one side
Nr days hospitalisation	5 (4-9)	4 (4-5)	<0.03 one side
Dose Salbutamol (ml)	40 (23-75)	35 (22-48)	<0.04 one side
Dose Ipratropium (drops)	240 (129-380)	173 (98-210)	<0.32
Neutrophil count (/mm)	10,972 (6,971-14,744)	8,012 (5,796-9,109)	<0.03 one side
Mean $\pm$ SD	10,894 $\pm$ 5,127	7,699 $\pm$ 3,107	
CRP (mg/ml)	1,1 (0,5-1,7)	0.7 (0.5-1,8)	<0.53
Mean $\pm$ SD	3.9 $\pm$ 7.2	1.6 $\pm$ 1.9	

Table 2: 52 patients (26 girls and 26 boys) with cystic fibrosis at 2(2y) and 5 (5y) years of age

<u>Females</u>	<u>Males</u>		<u>F>M</u>		
<u>Data</u>	<u>Median (p25-75)</u>		<u>Median (p25-75)</u>		<u>P(Mann</u>
<u>Whitney)</u>					
No. inf. at 2y	6	(4-7)	3.5	(2-4)	<0.015*
Age of first Inf	3	(2-5)	5	(4-11)	<0.04 (one
					side)
WBC/mm ³	10,780	(8,030 – 12,240)	10,015	(9,017 – 11,550)	<0.96
WBC/mm ³ 5y	12,160	(10,600 - 15,580)	8,910	(5,870 – 11,140)	<0.043
Neutro/mm ³ 2y	4,033	(2,384 – 5,617)	2,755	(1,975 – 4,074)	0.32
Neutro/mm ³ 5y	7,814	(5,789 – 9,703)	2,949	(2,653 – 4,311)	<0.010*
ESR mm/h 2y	7	(7 – 9)	12	(6 -14)	<0.22
ESR mm/h 5y	14	(10 – 16)	14.5	(8 – 29)	< 0.72
FEV1 (%) 5y	86	(66 – 96)	82	(82 – 83)	1.0
PercentileW5y	5	(3 – 17)	15	(7 – 50)	<0.21
Pseudom. 5y (%)	69 %		44%		
<0.033(CHsq one side)					

Table 3: 47 children (23 girls and 24 boys) with sickle cell anaemia

	<u>Girls (n=23)</u>	<u>Boys (n=24)</u>	<u>P</u> <u>(Mann Whitney)</u>
	Median (p25-75)	Median (p25-75)	
Age (months)	110 49-132	83 38-138	0.76
Total number of crises since diagnosis	4 2-6	2 1-3	< 0.01*
Number of ACS episodes Observed in the cohort	15	9	<0.04* (ChSq)
Age first symptoms	11 7-32	21 8-57	0.24
Correlation no. of crisis/ age	r = 0.48(p< 0.02*)	r = 0.08(NS)	
Correlation no. of days hosp /age	r = 0.42(p< 0.05)	r = 0.14(NS)	