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Journal of Cellular Immunology Short Communication

# Sex Differences in Clinical Characteristics and Platelet Activation in Respiratory Syncytial Virus Bronchiolitis

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**Received date:** April 29, 2020, **Accepted date:** May 29, 2020



Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis. It is a single-stranded RNA virus of the Paramyxoviridae family that is transmitted through nasopharyngeal or conjunctival mucosa from infected individuals. The incubation period ranges from 2 to 8 days [1]. Two antigenically different RSV subtypes exist, A and B. Although some studies have shown that RSV-A is associated with an increased disease severity [2], other studies have shown that, either RSV-B is more severe, or that the two subtypes have equivalent severity [3].

RSV induces upper and lower respiratory tract illness in children under 2 years of age worldwide and it is responsible for hospitalization in this age group in developed countries [4]. Frequently, it leads to severe morbidity and mortality, especially in premature infants and children with other chronic diseases such as thrombocytosis [5]. Furthermore, in terms of long-term complications, children who have contracted bronchiolitis during childhood are more likely to develop asthma in the following years [6].

Bronchiolitis is a seasonal pathology with an epidemic peak between December and January [7]. It is characterized by acute inflammation, edema and necrosis of epithelial cells lining small airways, increased mucus production and bronchospasm [8]. In acute RSV bronchiolitis, infection is quickly followed by inflammatory response, mediated by the innate immune system, and the release of numerous inflammatory cytokines, such as thrombopoietin, IL-6, IL-1alpha, IL-8, IL-11 and TNFα [9,10]. Moreover, inflammatory cells can generate both endogenous reactive oxidizing species (ROS) and reactive nitrogen species (RNS) contributing to increase plasma's total oxidative status that could play a role in bronchiolitis severity. In fact, an increase of total oxidative status has been found in moderate bronchiolitis, but not in mild bronchiolitis [11], and up-regulated levels of oxidative stress markers have been found in children's bronchoalveolar fluid with post-infectious bronchiolitis [12]. During inflammation, an increased platelet count (secondary to reactive thrombocytosis) often asymptomatic may occur in response to cytokine production. Literature data show that in bronchiolitis, thrombocytosis prevalence may range from 8.4% to 38.6%, with higher counts observed in RSV positive infections [13].

Platelets play an important role in antimicrobial host defense and tissue repair, but the mechanism utilized by the infection to induce thrombocytosis in patients with bronchiolitis has not yet been understood. Platelets have an intimate relationship with lungs where they are present in alveolar capillaries together with erythrocytes and

leukocytes. They likely have specialized activities in lung repair [14]. An increase of their number may represent a retrospective marker of viral infections indicating the severity of lower respiratory tract infections.

Although limited, there are literature data indicating sex differences in pediatric age. Statistical data do not explain whether the cause of these differences is due to genetic, metabolic, hormonal or environmental factors. Indeed, sex has a major impact on outcomes from a range of infectious diseases, starting from the beginning of life.

Referring to bronchiolitis, several studies have shown that, as in many other viral infections, the incidence is higher in boys than in girls [15-17]. This difference seems based on the girls' development of an immune response, both humoral and cell-mediated, which on one side protects from infections, and on the other side exposes them to a greater risk to develop autoimmune and inflammatory pathologies.

Recently we published, on the *Italian Journal of Pediatrics*, data which came from a retrospective study conducted on patients admitted with bronchiolitis in the period from January to December 2017 to Bambino Gesù Children's Hospital of Rome (Italy) [18]. This study was aimed to investigate if, during RSV infection, sex can affect the clinical characteristics of children and also if platelets have a role during infection. In particular, we selected only patients (112 boys and 91 girls) aged 12 months or less, at their first episode of bronchiolitis. Conversely, we excluded from the study patients infected by unknown viruses, with history of prematurity, immunodeficiency or with congenital heart diseases.

On the basis of the differences between the glycoprotein G present on the viral capsid, two antigenic groups of RSV have been analyzed: group A (RSVA) and group B (RSVB). The most common virus detected in these patients was RSVB (in 58% of boys and in 47% of girls), followed by RSVA (in 11.6% of boys and in 16% of girls).

These data highlight the influence of sex in the clinical course of bronchiolitis. In particular, a significant ($p=0.030$) sex difference in RSVB infections and C reactive protein (CRP) values was found. Specifically, CRP values were higher in girls than boys (1.11 mg/dL *vs* 0.92 mg/dL respectively; $p<0.05$). Furthermore, we found that the use of cortisone was significantly different in the two sexes ($p=0.05$). Cortisone therapy was used in 46.4% of the boys and 60% of the girls. No significant differences were detected in the oxygen and aerosol therapies. Also, sex differences in the count of platelets were found during the hospitalization. Girls developed a mild thrombocytosis more frequently than boys (90% of girls *vs* 78.3% of boys; $p = 0.01$), while severe thrombocytosis was observed in 21.7% of boys and 10% of girls; $p=0.05$). As mentioned above, platelets play an important role in anti-microbial host defense and their abundance can result in a hyper-coagulable state or thrombogenesis [19]. Based on this, to define the role of platelets during RSV infections, we selected a group of patients with diagnosis of moderate bronchiolitis (15 boys and 12 girls) admitted from January to March 2018. We chose patients affected by moderate bronchiolitis because in this form of the disease, the oxygen support is delivered with low flow rates. Moreover, the only patients that were included were the ones infected by RSVB, the most common cause of bronchiolitis in both male and female infants frequently causing severe morbidity and mortality [5]. Interestingly, we found that sex differences occurred in: i) platelet activation, evaluated in term of phosphatidylserine externalization; ii) platelet homotypic aggregation, evaluated in term of positivity to PAC-1; and iii) platelet heterotypic aggregation, evaluated in term of surface expression of P-selectin (CD62). In particular, we found that in RSVB bronchiolitis, with respect to girls, boys: i) show higher, although not significant, ROS levels in blood; ii) have a higher percentage of activated platelets (7.8% *vs* 2%; $p<0.05$); and iii) have a higher number of platelets forming homotypic aggregates (2.5% *vs* 0.7%; $p<0.05$). Conversely, we found that girls have a higher percentage of platelets forming heterotypic aggregated (35% vs 24%; $p<0.05$) than boys. These data support the hypothesis that activated platelets in children's blood with RSV bronchiolitis could contribute, in a sex-dependent manner, to thrombocytosis. In particular, we hypothesize that a sort of "hyper-aggregation" of platelet could determine an increased thromboembolic complication in girls with bronchiolitis due to this significant production of heterotypic aggregates.

In summary, we can assume that viral infection activates lung's microvascular endothelial cells, leading to increased expression of endothelial ligands capable of engaging platelet receptors [20-22]. Specifically, we suppose that the platelets linked to the endothelium by the integrin GP αIIbβ3 (evaluated with PAC-1 positivity) contribute to the formation of microthrombi. The highest percentage of PAC-1 positive platelets in boys with bronchiolitis would explain the greatest complication in boys than in girls.

## Acknowledgments

We thank Carlotta Catalano for revising English.

## Sources of Funding

This study was in part supported by the Italian Ministry of Health (RF-2013-02358715 to E.S.).

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