Immune screening in children with Down Syndrome

1 | BRIEF REPORT

Children with Down Syndrome (DS) are an immunodeficient cohort with decreased T and B cell counts, suboptimal response to vaccinations, impaired neutrophil chemotaxis, and dysregulated cytokine responses. 1 Children with DS are at increased risk of infections, particularly recurrent respiratory tract infections (RRTIs), otitis media, and pneumonia, 2 with more frequent hospitalisations. 3 We hypothesised that given the abnormalities in immune screening tests of children with DS, there are potential biomarkers that could allow us to predict clinical outcomes and focus care for higher risk children.

Down syndrome children who were clinically well with no recent evidence of fever or infection were recruited from the dedicated paediatric DS clinic. RRTIs were defined as ≥3 episodes of lower respiratory tract symptoms; bronchitis, bronchiolitis, or pneumonia per year. 2 A 17.07% (n = 28/164) of the cohort had previous surgical intervention for congenital heart disease, but all were >3 months post-operative when recruited. Patient blood samples (3 ml) were collected for immune screening for CD3+, CD4+, CD8+, CD19+, CD56+, Ig (immunoglobulin)M, IgG, IgA, and antibody titres to pneumococcus and tetanus and full blood counts (FBC); and were compared to established and non-hospitalised sub-groups (p = 0.32; Table 1).

Using binary logistic regression models there was a significant association between low WCC and the clinical outcome (RTI) of ever requiring hospitalisation (p = 0.015). Examining this further, a WCC ROC curve (Figure S4A) had an area under the curve of 0.669 [95% CI 0.563–0.774; p = 0.002]. Neutrophils had similar statistical significance, with a low count more likely to have occurred in those previously hospitalised (AUC 0.668 [95% CI 0.568–0.771]; p = 0.002) (Figure S4B). The clinical outcome measure of RRTIs was associated with a significantly higher hospitalisation rate in the past (odds ratio 3.6, 95% CI 1.8–7.6).

A total of 82 children with DS had T and B lymphocyte subset analysis performed. CD3+ and CD4+ T lymphocyte counts were low in almost half of the patients. CD8+ cytotoxic T cell counts were decreased in 34.1%, normal in 61%, and high in 4.9%. Natural killer (CD56+) counts were low in 15.9%, normal in 79.3%, and increased in 4.9%. The B lymphocytes (CD19+) were low in 76.8% and within the normal range in 23.2% (Figure S1 and Table 1 show age-stratified results).

There were 112 children with DS who had immunoglobulin levels checked. The majority had normal IgM, IgG, and IgA in serum. IgM levels were low in 3.9% of children, IgG was low in 1.9%, and lastly IgA was reduced in 2.7% of the children with DS (Figure S1). There were 61 children with DS who had antibody titres to pneumococcus and tetanus. Regarding total pneumococcal Ig, 1.7% had low levels and 98.3% had acceptable titres, and for pneumococcal Ig2, 11.5% had low antibody titres, while 88.5% had acceptable levels. The tetanus titres were low in 3.3% and normal in 96.7% (Figure S1).

We demonstrated significant differences in WCC differentials and T and B cell subsets in children with DS compared to age-matched normative values, in keeping with the literature. 3 Almost 25% of children with DS had reduced WCC and lymphocyte counts, and 12% had lower neutrophil levels. The most striking deficiency

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**Table 1.** Children previously hospitalised had significantly lower WCC than those who had never been admitted (p = 0.009). Neutrophil counts were significantly lower in children with previous admissions with pneumonia versus those never hospitalised (p = 0.02). There were no differences in mean lymphocyte counts between hospitalised and non-hospitalised sub-groups (p = 0.32; Table 1).

**Abbreviations:** CHI, children’s Health Ireland; DS, Down Syndrome; FBC, full blood counts; Ig, immunoglobulin; RRTIs, recurrent respiratory tract infections; WCC, white cell count.

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| Patient cohort (n = 164) | Mean | Standard deviation |
|-------------------------|------|--------------------|
| Age                     | 5.22 | 4.34               |

| Age Subgroups | n   | Percentage (%) |
|---------------|-----|----------------|
| <1 years      | 31  | 18.9           |
| 1–5 years     | 62  | 37.8           |
| 5–10 years    | 45  | 27.4           |
| >10 years     | 26  | 15.9           |

| Hospitalisations | n   | Percentage (%) |
|------------------|-----|----------------|
| One admission for RTI | 56  | 37.1           |
| Multiple admissions for RTIs | 27  | 17.8           |

| Available Information | n = 151 (92%) | Percentage (%) |
|-----------------------|---------------|----------------|
| Self-reported RRTIs   | 57            | 34.7           |

| Hospitalisation stratification | n   | Mean (x10⁹/l) | Standard deviation | Standard error | Mean | p-Value |
|-------------------------------|-----|--------------|--------------------|---------------|------|---------|
| White cell count Hospital     | 42  | 5.48         | 1.99               | 0.31          | 0.009|
| No Hospital                   | 78  | 6.78         | 2.80               | 0.32          |      |
| Neutrophils Hospital          | 42  | 2.53         | 1.31               | 0.20          | 0.02 |
| No Hospital                   | 78  | 3.51         | 2.61               | 0.30          |      |
| Lymphocytes Hospital          | 42  | 2.30         | 1.01               | 0.16          | 0.33 |
| No Hospital                   | 78  | 2.47         | 0.91               | 0.10          |      |

| Low white cell counts by age stratification | n   | Percentage (%) |
|--------------------------------------------|-----|----------------|
| <1 years                                   | 9   | 27.3           |
| 1–5 years                                  | 10  | 16             |
| 5–10 years                                 | 15  | 34.1           |
| >10 years                                  | 6   | 23.8           |

| Low neutrophils counts by age stratification | n   | Percentage (%) |
|--------------------------------------------|-----|----------------|
| <1 years                                   | 11  | 36.4           |
| 1–5 years                                  | 3   | 4              |
| 5–10 years                                 | 8   | 17.1           |
| >10 years                                  | 3   | 9.5            |

| Low lymphocyte counts by age stratification | n   | Percentage (%) |
|-------------------------------------------|-----|----------------|
| <1 years                                   | 7   | 22.2           |
| 1–5 years                                  | 13  | 20.5           |
| 5–10 years                                 | 9   | 20             |
| >10 years                                  | 9   | 33.3           |

| Low CD4+ counts by age stratification     | n   | Percentage (%) |
|-------------------------------------------|-----|----------------|
| <1 years                                   | 4   | 12.5           |
| 1–5 years                                  | 37  | 59.5           |
| 5–10 years                                 | 14  | 32             |
| >10 years                                  | 11  | 41.7           |

| Low NK cell counts by age stratification  | n   | Percentage (%) |
|------------------------------------------|-----|----------------|
| <1 years                                  | 4   | 12.9           |
| 1–5 years                                 | 20  | 32.2           |
| 5–10 years                                | 0   | 0              |
| >10 years                                 | 4   | 15.3           |
in lymphocyte subsets was seen in the CD19+ B cell, with over 75% of children with DS having reduced counts which have been widely corroborated in other studies. Over one-third of children with DS have had RRTIs, and 37% of this population were hospitalised at least once due to an RTI. Children with DS who had a low WCC or neutrophil count were significantly more likely to hospitalisation due to an RTI and the lower the counts the higher the probability of hospital admission.

Many studies have found mixed correlations between lymphocyte subsets and infection-related hospitalisations, though Martinez et al. found only Treg number and age correlated with infection-related admissions in a DS cohort. In our study, the CD19+ B cell was the most markedly reduced of all studied cells. Children with DS have reduced CD19+ B cells, and impaired B cell function with a reduction of switched memory B cells, and a reduced capacity for vaccination response, leading to a less robust immune response over time.

We report essentially normal immunoglobulin IgM, IgG, and IgA levels in children with DS with variability in the literature. Although children with DS can produce immunoglobulins, they seem suboptimal and may be unable to maintain long-term immunity. Children with DS are vulnerable to vaccine-preventable diseases and extra immunisations are required.

To the best of our knowledge, this is the first study to provide a link between WCC and a history of hospitalisation in children with DS. Further studies should corroborate our results, as identifying those children with DS who are at greatest risk with a simple, ubiquitous test could be key to improving the standard of care.

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CONFLICT OF INTEREST

None to disclose.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**TABLE 1 (Continued)**

| Low B cell counts by age stratification | N   | Percentage (%) |
|----------------------------------------|-----|----------------|
| <1 years                               | 17  | 54.8           |
| 1–5 years                              | 49  | 79             |
| 5–10 years                             | 38  | 84.4           |
| >10 years                              | 17  | 65.3           |

Note: WCC, neutrophils, and lymphocytes (x109/l) of children with DS who were previously hospitalised with pneumonia (“Hospital”), compared to those who were never hospitalised (“No Hospital”). p value < 0.05 denoted in bold.

Abbreviations: RRTI, recurrent respiratory tract infection; RTI, respiratory tract infection; WCC, white cell count.
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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.