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Natural immunity against *Haemophilus influenzae* type a and B-cell subpopulations in adult patients with severe chronic kidney disease

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ABSTRACT

Individuals suffering from severe chronic kidney disease (CKD) are immunocompromised and therefore highly susceptible to various infections including *Haemophilus influenzae* type a (Hia), an emerging pathogen in North American Indigenous populations. Immunocompromised Indigenous adults are considered a target for a new Hia vaccine under development. In an attempt to foresee their response to Hia immunization, we studied natural immunity against Hia and B-cell subpopulations in sixty patients with CKD residing in a geographic region with noticeable presence of Hia invasive disease. Serum bactericidal activity (SBA) against Hia, concentrations of IgG and IgM antibodies specific to Hia capsular polysaccharide, and B-cell subpopulations were studied in patients with CKD and 35 healthy controls of the same age. Of the patients with CKD, proportions and absolute numbers of B-cell subpopulations were determined for 28 patients. The patients had lower SBA titres compared to controls. Although no significant differences in anti-Hia IgG or IgM antibody concentrations between control and CKD groups were found, IgM antibody concentrations were higher in Indigenous than non-Indigenous patients. Patients with CKD had a higher proportion of B cells (CD19+), class switched memory B cells (CD19+CD27+IgM-) and a lower proportion of CD19+CD27-IgM- B cells compared to healthy controls. Non-Indigenous patients with CKD had significantly higher proportions of IgM memory B cells and CD19+CD27-IgM- B cells compared to Indigenous patients with no significant difference in absolute numbers. Because 72% of CKD patients had detectable SBA titres and 100% had detectable IgG and IgM antibodies it is possible that a portion of IgM memory B cells and class switched memory B cells are specific for Hia resulting from a natural exposure to the pathogen. The data suggest that a Hia-conjugate vaccine may be immunogenic in adult patients with CKD as it will potentially induce re-activation of immunological memory against Hia.

1. Introduction

Severe chronic kidney disease (CKD) is defined as a decreased kidney glomerular filtration rate (GFR) of less than 30 mL/min/1.73 m^2 for a minimum of 3 months, and is a serious condition characterized by high mortality rates [1,2]. Increased inflammatory responses and reactive oxygen species generation associated with uremia contribute to the development of other conditions, such as cardiovascular disease and cancer [3]. Infection is the second major cause of death in these patients after cardiovascular disease [4]. In patients with CKD, a high risk for septicemia and other severe infections is attributed to their compromised immune system and increased exposure to infectious agents in dialysis units [5,6]. In North America, CKD disproportionally affects Indigenous populations. The burden of CKD among Indigenous peoples is mostly influenced by dramatically rising incidence of obesity and type 2 diabetes mellitus resulting in diabetic nephropathy as well as a high rate of glomerulonephritis [7,8]. *Haemophilus influenzae* type a (Hia) is an emerging infection in North American Indigenous populations; invasive Hia disease mostly affects young children, the elderly, and immunocompromised individuals [9–11]. Our studies over the last 15 years have shown that invasive Hia disease is consistently present in Northwestern Ontario, Canada [12]. This geographic region has 25.4% population of Indigenous peoples [13], and several cases of invasive Hia disease occurred in Indigenous peoples suffering from CKD [11,14,15]. A new vaccine against Hia is currently under development in Canada [16]. Because immunocompromised individuals are highly susceptible to invasive Hia

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disease, patients with CKD are being considered as a target population for a new vaccine.

We have recently found that healthy Indigenous adults have higher titres of serum bactericidal antibodies against Hia compared to non-Indigenous peoples living in the same region suggesting that they may have developed immunological memory against Hia as a result of exposure to the pathogen [10,17]. While long-lived plasma cells are directly responsible for antibody production, memory B cells generated during the primary immune response are able to mount rapid recall response upon secondary exposure to the same antigen [18,19]. Therefore, the effect of adult immunization is critically dependent on the presence of memory B cells. As no previous research on memory B cells in Indigenous patients with CKD had been done, we made an attempt to characterize their subpopulations in Indigenous and non-Indigenous patients. We also tried to determine if there is an association between natural humoral immunity to Hia and B-cell subpopulations.

2. Methods

2.1. Participants

This study was approved by the research ethics boards of Lakehead University and the Thunder Bay Regional Health Sciences Centre (TBRHSC). Sixty patients with stage four or five CKD (stage 4, GFR < 30 mL/min/1.73 m^2, stage 5, GFR <15 mL/min/1.73 m^2) [2] who received hemodialysis in the TBRHSC were recruited from May 2015 – January 2018; all were required to provide informed consent before determining their eligibility for the study. Ethnicity was determined based on self-declaration. Patients with severe CKD were considered eligible if they: were over 18 years old, did not have a history of immunocompromising conditions, were not taking immunosuppressive medications for more than 14 days in the past 6 months, had not received any vaccine in the past month and were not receiving blood transfusions or blood products in the past 3 months. The majority of 35 healthy controls were recruited through the Thunder Bay 55 Plus Centre, and were considered eligible if they were over the age of 18 years old and did not have a history of taking immunosuppressive medications. Table 1 displays the demographics of study participants; supplementary Table 1 describes additional information on the underlying causes of CKD and comorbidities.

2.2. Analysis of B cells

Peripheral blood mononuclear cells were isolated using Lymphoprep (Stemcell Tecnologies, Vancouver, BC, CAN). Monocytes were removed using two consecutive incubations in complete culture medium, i.e. RPMI 1640 medium with L-glutamine (Fisher Scientific, Whitby, ON, CAN) and 1% antibiotic-antimicotic (Life Technologies, Burlington, ON, CAN) supplemented with 20% fetal bovine serum (FBS, Fisher Scientific) in a cell culture dish for 1 h at 37 °C, 5% CO_2. Non-adherent cells were then washed and re-suspended in complete culture medium supplemented with 10% FBS at 2×10^6 cells/mL. From the cell suspension, 200,000 cells were immunostained with PE Mouse Anti-Human CD19, APC Mouse Anti-Human IgM, and PerCP-Cy™5.5 Mouse Anti-Human CD27 at 4 °C for 1 h. Samples were analyzed with BD FACSCalibur™ Flow Cytometer and CELLQUEST PRO software (BD Biosciences) to determine proportions of B cells (CD19+) and subpopulations: naïve (CD27-IgM+), IgM memory (CD27+IgM+), CD27-IgM-, and class switched memory (CD27+IgM-) (Fig. 1). Purity of CD19+gated B cells was verified by counterstaining cells with FITC Mouse Anti-Human CD3 and PerCP-Cy™5.5 Mouse Anti-Human CD14 (BD Biosciences).

Analysis of B cells was conducted for 28 out of 60 patients (supplementary Table 2). For these patients, a complete blood count (CBC) was performed at the TBRHSC clinical lab to determine the lymphocyte count ($\times 10^9$ cells/L). The absolute number of B cells was calculated by multiplying the percentage of CD19+ cells of the total gated lymphocyte population by the total lymphocyte count. Similarly, the numbers of B-cell subpopulations were determined by multiplying the percentage of each subpopulation by the absolute number of B cells (supplementary Table 3).

2.3. Analysis of Haemophilus influenzae type a specific antibodies

Serum Bactericidal Assay (SBA) was performed as previously described [17] using Hia strain 08-191and baby rabbit complement (Pel-Freez, Rogers, AR, USA) as the exogenous complement source. SBA titers were determined as the reciprocal serum dilution required to kill $\geq$ 50% of the initial bacterial inoculum [20]. Titers below the lower detection limit of 16 were reported as 8 for statistical purposes.

IgG and IgM antibodies specific to Hia capsular polysaccharide were quantified by ELISA as previously described [10]. Antibody concentrations were determined using our internal standard that was standardized to the Hia reference serum provided by the Centers for Disease Control and Prevention [10]. The internal standard contained 1.25 μg/ mL anti-Hia IgG and 2.09 μg/ mL anti-Hia IgM. The standard curve was made using a log-log non-linear regression to produce the line of best fit. Outliers were deleted and values were considered acceptable if the coefficient of variation was below 30%.

2.4. Statistical analysis

Statistical analysis was performed using Graph-Pad Prism 5 (GraphPad Prism Software Inc., San Diego, CA). The means for the percentage of each B-cell subpopulation and absolute numbers of B cells were calculated as well as the geometric means for anti-Hia antibody concentrations and SBA titres with 95% confidence intervals (CI). Groups were compared either using a Student's *t*-test, or Mann-Whitney *U* test, one-way ANOVA, or Kruskal-Wallis test depending on data distribution and number of groups compared. Correlation between antibody concentrations and B-cell subpopulations was determined using Pearson or Spearman's cor-

Table 1
Demographics of study participants.

Group	*n*	Age (years) Mean ± SEM	Range	No. (%) Female	No. (%) Indigenous
Healthy Controls	35	62 ± 2	36–85	22 (63)	5 (14)
CKD Patients	60	61 ± 2	32–85	26 (43)	30 (50)
CKD Indigenous	30	53 ± 2^{+*}	32–80	15 (50)	
CKD non-Indigenous	30	70 ± 2*	43–85	11 (37)	

+p < 0.0001, chronic kidney disease (CKD) to healthy controls; *p < 0.01, Indigenous to non-Indigenous CKD patients (Student's *t*-test). The ages were significantly different between healthy controls and both CKD groups (Indigenous and non-Indigenous) (p < 0.0001, one-way ANOVA).

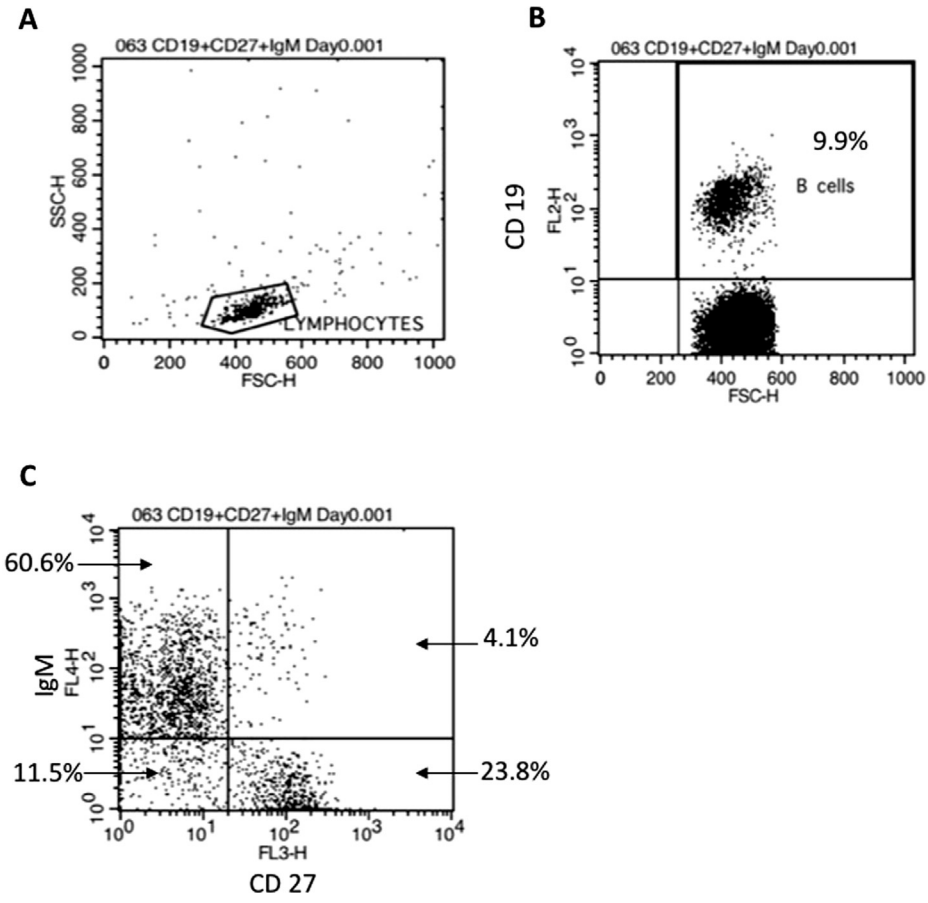

Fig. 1. Flow cytometry analysis. Proportions of naïve (CD27- IgM+), IgM memory (CD27+IgM+), CD27- IgM-, and class switched memory (CD27+IgM-) B cells were determined by first gating the lymphocyte population (A), then gating the CD19+lymphocytes (FL2) (B). These events were then separated based on their CD27 (FL3) and IgM (FL4) expression (C). The percent gated values for the proportions of B cell subpopulations were determined. One representative experiment is shown; CD19+ (9.9%), CD27-IgM+ (60.6%), CD27+IgM+ (4.1%), CD27-IgM- (11.5%), and CD27+IgM- (23.8%).

relation. Significance was determined at p value < 0.05. Outliers were identified and removed [21].

3. Results and discussion

To assess natural immunity against Hia in patients with severe CKD, we quantified functionally active antibodies using SBA, and anti-Hia capsular polysaccharide specific IgG and IgM using ELISA. The patients had lower geometric mean SBA titres than healthy controls (145, CI: 86.53–244.1 *vs.* 324.7, CI: 189.4–556.4, Student's *t*-test, p < 0.05, Fig. 2A). Moreover, larger proportion of patients had SBA titres below lower detection limits compared to healthy controls, 28% (17/60) *vs.* 9% (3/35), p < 0.05 (Fisher's exact test). These results are in agreement with our earlier observations suggesting that CKD may negatively affect natural immunity against Hia and therefore this patient population may be at risk of invasive Hia disease [10]. However, no significant difference in SBA titres between healthy controls, CKD Indigenous, and CKD non-Indigenous patients was detected when a multiple comparisons test was performed (Fig. 2A and B). No significant difference in anti-Hia capsular polysaccharide IgM (1.0 μg/ mL, CI: 0.8–1.3 *vs.* 1.2 μg/ mL, CI: 0.9–1.5) or IgG (0.6 μg/ mL, CI: 0.4–0.9 *vs.* 0.5 μg/ mL, CI: 0.4–0.7) concentrations between patients and controls was detected using either a Student's *t*-test or Mann-Whitney *U* test, or between healthy controls, CKD Indigenous, and CKD non-Indigenous using a multiple comparisons test (p > 0.05) (Fig. 2C–F).

While SBA geometric mean titres (GMT) in Indigenous CKD patients did not significantly differ from non-Indigenous patients (111.4, CI: 51.90–239.2 *vs.* 189.6, CI: 90.98–395.0, p > 0.05, Fig. 2B), the concentration of IgM antibodies specific to Hia capsular polysaccharide was significantly higher in Indigenous compared to non-Indigenous patients (1.4 μg/mL, CI:1.0–1.9 *vs.* 0.7 μg/mL, CI: 0.5–1.1, Mann-Whitney *U* test, p < 0.05, Fig. 2D). No difference in specific IgG concentrations between these groups was detected (Fig. 2F).

In all the groups, the geometric mean concentrations of Hia capsular polysaccharide-specific IgM were higher than the concentrations of specific IgG (Fig. 2C–F). Healthy controls had a slightly greater ratio of IgM to IgG compared to patients with CKD (2.4:1 *vs.* 1.7:1, p > 0.05, Fig. 2C–E) while the Indigenous and non-Indigenous patients had similar ratios (1.8:1 *vs.* 1.4:1, p > 0.05, Fig. 2D–F). These results confirm our previous findings on the prevalence of IgM in the anti-Hia capsular polysaccharide antibody repertoire and higher anti-Hia IgM concentrations in Indigenous than non-Indigenous adults [10,17]. Although the prevalence of IgM over IgG suggests that anti-Hia capsular polysaccharide antibodies may be part of the natural IgM antibody repertoire, this may also be an indicator of a recent exposure to the pathogen [17]. Indeed, our epidemiological studies have found that during the last two decades, Hia has been consistently present in Indigenous communities of this geographic region [11,14,15]. Naturally acquired antibodies are largely determined by the colonization, and the colonization rates may vary among different populations depending on age, race, living conditions, and socio-economic status [22,23]. However, the relationship between the development of naturally acquired antibodies and *H. influenzae* colonization history

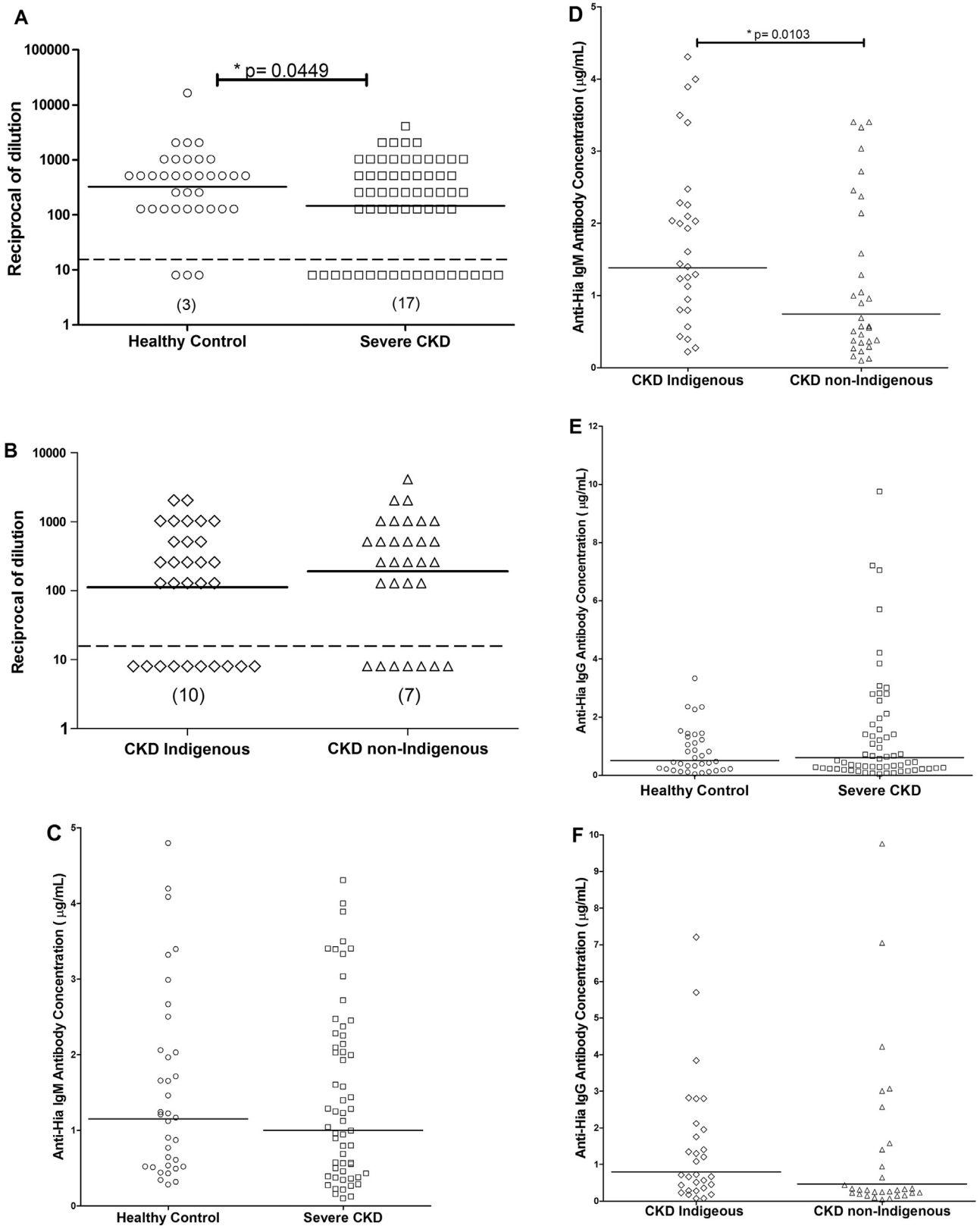

Fig. 2. Serum bactericidal antibody against *Haemophilus influenzae* type a in healthy controls and CKD patients (A) and Indigenous *vs*. non-Indigenous CKD patients (B). The antibody titre was defined as the reciprocal of serum dilution resulting in $\geq$ 50% killing of Hia strain 08–191. The solid line indicates geometrical mean titre (GMT). The number of individual samples below the lower limit of detection (dashed line) is indicated on the graph. $^{*}p < 0.05$, Student's *t*-test. The geometric mean concentrations (GMC, solid line) of anti-Hia capsular polysaccharide IgM (C-D) and IgG (E-F) in healthy controls and CKD patients (C, E) and Indigenous *vs*. non-Indigenous CKD patients (D, F). $^{*}p < 0.05$, Mann-Whitney *U* test. No significant difference between the 3 groups (healthy control, CKD Indigenous, and CKD non-Indigenous) for GMT or GMC (IgM or IgG) was detected by one-way ANOVA or Kruskal-Wallis test.

is complex, and potential effects of some cross-reactive antigens present in the environment need to be considered [17].

Severe CKD is associated with chronic inflammation [24]. In other inflammatory diseases, such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease, B-cell subpopulations are often altered compared to healthy people [25]. This suggests that patients with CKD may also have alterations in B-cell subpopulations. To determine proportions of B cells and their subpopulations, we have used flow cytometry analysis to detect the cell surface expression of CD19 as a marker for B cells and CD27 for memory B cells (Fig. 1). IgM memory B cells and switched memory B cells were defined as CD19+CD27+IgM+ and CD19+CD27+IgM- cells, respectively [26].

Our analysis showed that patients with CKD had a higher percentage of B cells in comparison to healthy controls (11%, CI: 5.9–8.0 *vs.* 7%, CI: 8.4–13.7, Student's *t*-test, $p < 0.01$, Table 2), with no significant differences between Indigenous and non-Indigenous patients ($p > 0.05$, Table 2). There was a significant difference in the proportions of B cells between healthy controls, CKD Indigenous, and CKD non-Indigenous patients (one-way ANOVA, $p < 0.01$). Higher proportion of B cells in patients with CKD compared to healthy controls is likely due to chronic inflammation caused by uremia, and can be associated with common co-morbidities found in these patients, such as diabetes mellitus and chronic obstructive pulmonary disease [27,28]. We also found that patients with CKD had significantly higher proportions of class switched memory B cells (CD19+CD27+IgM-) (22%, CI: 16.4–28.5 *vs.* 16%, CI: 12.2–19.1, Student's *t*-test, $p < 0.05$) and lower proportions of CD19+CD27-IgM- B cells (16%, CI: 13.1–19.2 *vs.* 20%, CI: 16.4–28.5, Mann-Whitney *U* test, $p < 0.05$) compared to the controls (Fig. 3A and B). When B-cell subpopulations were compared between healthy controls, CKD Indigenous and CKD non-Indigenous patients, no significant differences between the groups were found except for CD19+CD27-IgM- B cells (Kruskal-Wallis test, $p < 0.001$).

The T-cell-dependent antibody response is characterized by the generation of class switched memory B cells [18]. Upon re-exposure to the same antigen, these cells are able to respond rapidly resulting in the production of more antigen specific memory B cells as well as plasma cells that produce high affinity antibodies [19]. An increase in proportion of class switched memory B cells in CKD patients could suggest that such patients have been exposed to pathogens more often than healthy controls. Indeed, patients with CKD undergoing hemodialysis spend a lot of time in close proximity to other patients in the hospital environment, and experience frequent disruption of the skin barrier from needles or catheter ports. As part of the standard clinical practice these patients receive vaccines prescribed for high-risk adult populations (against pneumococcal, hepatitis B virus, and varicella zoster infections) that can potentially increase the population of class switched memory B cells.

It is more difficult to interpret a decrease in the proportion of CD19+CD27-IgM- because these cells are not well characterized in the literature. Similarly to these cells, the origin and functional roles of Double Negative (DN) B cells (CD19+CD27-IgD-) are uncertain. Because DN B cells accumulate in the elderly, it was suggested that these cells are the exhausted terminally differentiated memory B cells [29–31]. Accordingly, a higher proportion of CD19+CD27-IgM- B cells (18%, CI: 13.4–22.6 *vs.* 13%, CI: 10.8–13.5, Mann-Whitney *U* test, $p < 0.05$) in non-Indigenous compared to Indigenous patients (Fig. 3D) may be due to the fact that the non-Indigenous patients were significantly older (69 ± 3 *vs.* 54 ± 3 years, Student's *t*-test, $p < 0.01$, supplementary Table 2).

We have also observed higher proportions of IgM memory B cells in non-Indigenous compared to Indigenous patients (12%, CI: 7.1–16.24 *vs.* 7%, CI: 4.3–9.5, Student's *t*-test, $p < 0.05$) (Fig. 3C). The IgM memory B cells are generated in the process of the T cell-independent immune response and also increase with age [32–34] that may explain their higher proportion in the non-Indigenous group of CKD patients. It is tempting to speculate that higher IgM anti-Hia antibody concentrations found in Indigenous compared to non-Indigenous patients may potentially be associated with an increased proportion of IgM memory B cells, as previous studies demonstrated an essential role of this subpopulation in antibody response to bacterial capsular polysaccharides [26]. However, our analysis did not reveal any noticeable correlation between B-cell subpopulations and antibody levels or SBA titres (data not shown). It is not surprising because antigen-specific B cells represent a very small fraction of the total B cell population. For example, in healthy unimmunized adults, only 0.5% of total CD19+ B cells are specific to any individual pneumococcal polysaccharide antigen [35].

For the interpretation of changes in B-cell subpopulations found in our patients, it is necessary to consider that the total numbers of B-lymphocytes may be decreased in CKD patients [36]. We calculated the absolute numbers of B cells and their subpopulations for 28 CKD patients (supplementary Table 3). No significant differences between Indigenous and non-Indigenous patients for absolute number of any of the subpopulations were found (supplementary Table 3). Because the lymphocyte counts were not available in our control group we compared the absolute numbers of B cells and their subpopulations in CKD patients with published data (supplementary Table 4a and b). The analysis indicated that approximately 60% of our patients (16/28) had total lymphocyte counts within normal limits defined by the Medical Council of Canada ($1.0–4.0 \times 10^9$ cells/L) [37], and the remaining 12 had the counts below 1.0×10^9 cells/L. In comparison to absolute values of B-cell subpopulations in healthy adults between the ages of 26 and 50 reported by Morbach et al. [38], our patients had lower numbers of total lymphocytes, total B cells, naïve B cells, and IgM (non-switched) memory B cells, but similar numbers of class switched memory B cells and higher numbers of CD19+CD27-IgM- compared to reported DN cells (supplementary Table 4b). However, this comparison needs to be taken with caution because of age differences between our patients and their cohort of healthy adults (69 ± 3 *vs.* 54 ± 3 years, $p < 0.01$), as well as the use of different markers for defining naïve and IgM memory B cells (IgD+ rather than IgM+). Nevertheless, 93% (26/28 patients) of our CKD patients had numbers of B cells below the normal values reported by Morbach et al. (199 cells/μL, age 26–50 years) [38] and Qin et al. 2016 (216 cells/ μL, age 19–80 years) [39]. Similar to our findings, a previous study by Pahl et al. (2010) demonstrated that patients with end-stage renal disease had significantly reduced numbers of total B cells and their subpopulations including memory (CD19+CD27+) and naïve B cells (CD19+CD27-) in comparison to healthy controls [40]. In that study, a decrease in the absolute numbers of B cells was found to be associated with a decrease in BAFF receptor expression in transitional B cells despite elevated levels of IL-7 and BAFF in plasma. Considering that BAFF is a key differentiation and survival factor for B cells, the authors concluded that B-cell

Table 2
Percentage of CD19+lymphocytes determined by flow cytometry.

Group	Percentage of B cells (95% CI)
Healthy Controls	7.0 (5.9–8.0)
CKD	11.1 (8.4–13.7)**
CKD Indigenous	11.6 (7.7–15.4)**
CKD non-Indigenous	10.3 (6.2–14.3)*

Comparison of the mean proportions of B cells between patients with chronic kidney disease (CKD) to healthy controls (*$p < 0.05$, **$p < 0.01$, Student's *t*-test). The proportions of B cells were significantly different between healthy controls and both CKD groups (Indigenous and non-Indigenous) ($p < 0.01$, one-way ANOVA).

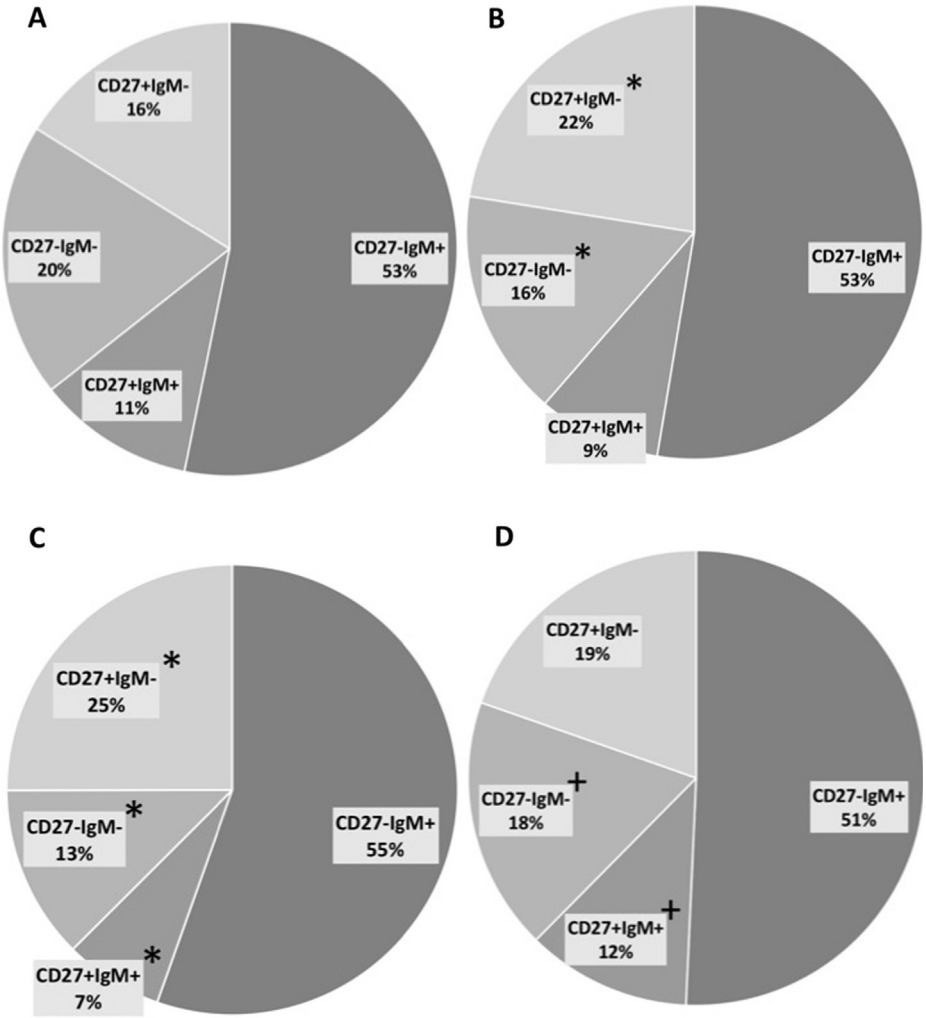

Fig. 3. Proportions of B cell subpopulations were determined by flow cytometry. Isolated peripheral blood mononuclear cells were immunostained for CD19, CD27, and IgM. Naïve (CD19+CD27- IgM+), IgM memory (CD19+CD27+IgM+), CD19+CD27- IgM-, and class switched memory B cells (CD19+CD27+IgM-) were determined for healthy controls (A), patients with chronic kidney disease (CKD) (B), CKD Indigenous (C) and CKD non-Indigenous (D). *$p < 0.05$ when comparing the CKD groups to healthy controls; +$p < 0.05$ comparing Indigenous and non-Indigenous patients with CKD (Student's *t*-test or Mann-Whitney *U* test). No significant differences between healthy controls, CKD Indigenous and CKD non-Indigenous patients were detected for any subpopulation except for CD19+CD27-IgM- cells, $p < 0.001$ (Kruskal-Wallis test).

lymphopenia in end-stage renal disease is caused by the impact of uremia on maturation of transitional B cells [40].

Because the majority of our patients had below normal absolute numbers of B cells, they may potentially exhibit a low response to immunization. Indeed, it was previously found that some CKD patients were hypo-responsive to pneumococcal polysaccharide vaccine [41]. The response rates to hepatitis B vaccination in these patients are also lower in comparison to healthy controls [42]. However, we have recently found that the pediatric *Haemophilus influenzae* type b (Hib) polysaccharide-protein conjugate vaccine is highly immunogenic in a group of adult patients with CKD, which is comparable to the one in present study [43]. Because the proposed Hia vaccine is designed based on the same principles as Hib-conjugate vaccine, i.e. composed of the capsular polysaccharide conjugated to a protein carrier, this may predict its sufficient immunogenicity in CKD patients.

This study has several limitations. There is a nearly 20-year difference between the mean ages of the CKD Indigenous and non-Indigenous patients (Student's *t*-test, $p < 0.01$). This difference reflects the demographics of adults suffering from CKD in Canada, i.e. people of Indigenous descent tend to develop CKD at a younger age [7]. For logistic reasons, we were unable to include a group of healthy Indigenous participants as a control and hence could not determine their B-cell subpopulations, although the data of serum Hia antibody levels and serum bactericidal activity in healthy Indigenous adults collected by our group in the same region and using the same methodology are available [10,17]. We did not study *H. influenzae* colonization rates in study participants although our ongoing studies indicate that approximately 8% of 3–5 year old First Nations children carry Hia in the nasopharynx (unpublished observations). However, the development of natural immunity to common bacteria such as *H. influenzae* is a result of multiple exposures to antigens. Given the transient pattern of *H. influenzae* colonization we did not consider data on single-point carriage in adults to be of value regarding the understanding of natural immunity to Hia and we were unable to conduct a longitudinal study of carriage in our cohorts for logistic reasons. Because we were unable to determine absolute lymphocyte numbers in healthy controls we have used the relevant data from literature for analysis. Lastly, no correction was applied to the threshold of statistical significance despite the many comparisons that were made, most involving small differences between groups.

4. Conclusion

Patients suffering from severe CKD may be at an increased risk for invasive Hia disease because they have decreased titres of serum bactericidal antibodies against Hia and reduced absolute numbers of B lymphocytes. Yet, 72% of CKD patients exhibited detectable serum bactericidal activity against Hia and 100% had detectable Hia-specific IgG and IgM. However, the minimum protective antibody concentrations against Hia are still unknown, and we must also consider that patients with chronic disease, such as those with CKD might require higher antibody concentrations for protection. Considering the presence of an increased proportion of class switched memory B cells in CKD compared to healthy controls, a fraction of memory B cells resulting from a natural exposure to the pathogen can be specific for Hia. The data would suggest that a new Hia-conjugate vaccine under development may be immunogenic in adult patients with CKD, as it will potentially induce re-activation of immunological memory specific to Hia.

Declaration of Competing Interest

All authors declare there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.036.

References

[1] Webster AC, Nagler EV, Morton RL, Masson P. Chronic kidney disease. Lancet 2017;389(10075):1238–52. https://doi.org/10.1016/S0140-6736(16)32064-5. PubMed PMID: 27887750. Pubmed Central PMCID.

[2] Levin A, Hemmelgarn B, Culleton B, Tobe S, McFarlane P, Ruzicka M, et al. Guidelines for the management of chronic kidney disease. CMAJ 2008;179 (11):1154–62. https://doi.org/10.1503/cmaj.080351. PubMed PMID: 19015566. Pubmed Central PMCID: PMC2582781..

[3] Mihai S, Codrici E, Popescu ID, Enciu AM, Albulescu L, Necula LG, et al. Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. J Immunol Res 2018;2018:2180373. https://doi.org/10.1155/2018/2180373. PubMed PMID: 30271792. Pubmed Central PMCID: PMC6146775.

[4] Naqvi SB, Collins AJ. Infectious complications in chronic kidney disease. Adv Chronic Kidney Dis 2006;13(3):199–204. https://doi.org/10.1053/j.ackd.2006.04.004. PubMed PMID: 16815225. Pubmed Central PMCID.

[5] Vaziri ND, Pahl MV, Crum A, Norris K. Effect of uremia on structure and function of immune system. J Ren Nutr 2012;22(1):149–56. https://doi.org/10.1053/j.jrn.2011.10.020. PubMed PMID: 22200433. Pubmed Central PMCID: PMC3246616.

[6] Liakopoulos V, Roumeliotis S, Gorny X, Dounousi E, Mertens PR. Oxidative stress in hemodialysis patients: a review of the literature. Oxid Med Cell Longev 2017;2017:3081856. https://doi.org/10.1155/2017/3081856. PubMed PMID: 29138677. Pubmed Central PMCID: PMC5613374..

[7] Ferguson TW, Tangri N, Tan Z, James MT, Lavallee BDA, Chartrand CD, et al. Screening for chronic kidney disease in Canadian indigenous peoples is cost-effective. Kidney Int 2017;92(1):192–200. https://doi.org/10.1016/j.kint.2017.02.022. PubMed PMID: 28433383. Pubmed Central PMCID.

[8] Dyck RF, Tan L. Non-diabetic end-stage renal disease among Saskatchewan aboriginal people. Clin Invest Med 1998;21(1):33. PubMed PMID: 9512883. Pubmed Central PMCID.

[9] Ulanova M, Tsang RSW. Haemophilus influenzae serotype a as a cause of serious invasive infections. Lancet Infect Dis 2014;14(1):70–82. https://doi.org/10.1016/S1473-3099(13)70170-1. PubMed PMID: 24268829. Pubmed Central PMCID.

[10] Nix EB, Williams K, Cox AD, St Michael F, Romero-Steiner S, Schmidt DS, et al. Naturally acquired antibodies against Haemophilus influenzae type a in Aboriginal adults, Canada. Emerg Infect Dis 2015;21(2):273–9. https://doi.org/10.3201/eid2102.140722. PubMed PMID: 25626129. Pubmed Central PMCID: PMC4313637..

[11] Kelly L, Tsang RS, Morgan A, Jamieson FB, Ulanova M. Invasive disease caused by Haemophilus influenzae type a in Northern Ontario First Nations communities. J Med Microbiol. 2011;60(Pt 3):384–90. https://doi.org/10.1099/jmm.0.026914-0. PubMed PMID: 21148281. Pubmed Central PMCID.

[12] Tsang RSW, Ulanova M. The changing epidemiology of invasive Haemophilus influenzae disease: Emergence and global presence of serotype a strains that may require a new vaccine for control. Vaccine 2017;35(33):4270–5. https://doi.org/10.1016/j.vaccine.2017.06.001. PubMed PMID: 28666758. Pubmed Central PMCID.

[13] Census Profile. 2016 Census, Northwest Ontario and Ontario [Internet]. 2016 [cited January 21, 2019]. Available from: <http://www12.statcan.gc.ca/census-recensement/2016/dp-pd/prof/details/page.cfm?Lang=E&Geo1=ER&Code1=3595&Geo2=PR&Code2=35&Data=Count&SearchText=Northwest&SearchType=Begins&SearchPR=01&B1=All&GeoLevel=PR&GeoCode=3595&TABID=1>.

[14] Brown VM, Madden S, Kelly L, Jamieson FB, Tsang RS, Ulanova M. Invasive Haemophilus influenzae disease caused by non-type b strains in Northwestern Ontario, Canada, 2002-2008. Clin Infect Dis 2009;49(8):1240–3. https://doi.org/10.1086/605671. PubMed PMID: 19761408. Pubmed Central PMCID.

[15] Eton V, Schroeter A, Kelly L, Kirlew M, Tsang RSW, Ulanova M. Epidemiology of invasive pneumococcal and Haemophilus influenzae diseases in Northwestern Ontario, Canada, 2010–2015. Int J Infect Dis 2017;65:27–33. https://doi.org/10.1016/j.ijid.2017.09.016. PubMed PMID: 28951105. Pubmed Central PMCID.

[16] Cox AD, Barreto L, Ulanova M, Bruce MG, Tsang R, Conference c. Developing a vaccine for Haemophilus influenzae serotype a: Proceedings of a workshop. Can Commun Dis Rep 2017;43(5):89–95. PubMed PMID: 29770071. Pubmed Central PMCID: PMC5764720.

[17] Nix EB, Choi J, Anthes C, Gaultier GN, Thorgrimson J, Cox AD, et al. Characterization of natural bactericidal antibody against Haemophilus influenzae type a in Canadian First Nations: a Canadian Immunization Research Network (CIRN) Clinical Trials Network (CTN) study. PLoS ONE 2018;13(8). https://doi.org/10.1371/journal.pone.0201282. PubMed PMID: 30110339. Pubmed Central PMCID..

[18] Kurosaki T, Kometani K, Ise W. Memory B cells. Nat Rev Immunol 2015;15 (3):149–59. https://doi.org/10.1038/nri3802. PubMed PMID: 25677494. Pubmed Central PMCID.

[19] Inoue T, Moran I, Shinnakasu R, Phan TG, Kurosaki T. Generation of memory B cells and their reactivation. Immunol Rev 2018 May;283(1):138–49. https://doi.org/10.1111/imr.12640. PubMed PMID: 29664566. Pubmed Central PMCID.

[20] Rouphael NG, Satola S, Farley MM, Rudolph K, Schmidt DS, Gomez-de-Leon P, et al. Evaluation of serum bactericidal antibody assays for Haemophilus influenzae serotype a. Clin Vaccine Immunol 2011 Feb;18(2):243–7. https://doi.org/10.1128/CVI.00219-10. PubMed PMID: 21177919. Pubmed Central PMCID: PMC3067367.

[21] William-Mendenhall RB, Beaver Barbara, Ahmed S. Introduction to probability and statistics. 3rd Canadian ed. Nelson Education; 2014.

[22] Wenger JD. Epidemiology of Haemophilus influenzae type b disease and impact of Haemophilus influenzae type b conjugate vaccines in the United States and Canada. Pediatr Infect Dis J 1998;17(9 Suppl):S132–6. PubMed PMID: 9781746. Pubmed Central PMCID..

[23] Scott JR, Millar EV, Lipsitch M, Moulton LH, Weatherholtz R, Perilla MJ, et al. Impact of more than a decade of pneumococcal conjugate vaccine use on carriage and invasive potential in Native American communities. J Infect Dis 2012;205(2):280–8. https://doi.org/10.1093/infdis/jir730. PubMed PMID: 22128315. Pubmed Central PMCID: PMC3244367.

[24] Qian Q. Inflammation: a key contributor to the genesis and progression of chronic kidney disease. Contrib Nephrol 2017;191:72–83. https://doi.org/10.1159/000479257. PubMed PMID: WOS:000435366800008. Pubmed Central PMCID. English.

[25] Miyagaki T, Fujimoto M, Sato S. Regulatory B cells in human inflammatory and autoimmune diseases: from mouse models to clinical research. Int Immunol 2015;27(10):495–504. https://doi.org/10.1093/intimm/dxv026. PubMed PMID: WOS:000364767100005. Pubmed Central PMCID. English.

[26] Kruetzmann S, Rosado MM, Weber H, Germing U, Tournilhac O, Peter HH, et al. Human immunoglobulin M memory B cells controlling Streptococcus

pneumoniae infections are generated in the spleen. J Exp Med. 2003;197 (7):939–45. https://doi.org/10.1084/jem.20022020. PubMed PMID: 12682112. Pubmed Central PMCID: PMC2193885.

[27] Muriach M, Flores-Bellver M, Romero FJ, Barcia JM. Diabetes and the brain: oxidative stress, inflammation, and autophagy. Oxid Med Cell Longev 2014;2014. https://doi.org/10.1155/2014/102158. PubMed PMID: 25215171. Pubmed Central PMCID: PMC4158559.

[28] Polverino F, Seys LJ, Bracke KR, Owen CA. B cells in chronic obstructive pulmonary disease: moving to center stage. Am J Physiol Lung Cell Mol Physiol 2016;311(4):L687–95. https://doi.org/10.1152/ajplung.00304.2016. PubMed PMID: 27542809. Pubmed Central PMCID: PMC5142126.

[29] Colonna-Romano G, Bulati M, Aquino A, Pellicano M, Vitello S, Lio D, et al. A double-negative (IgD-CD27-) B cell population is increased in the peripheral blood of elderly people. Mech Ageing Dev 2009;130(10):681–90. https://doi.org/10.1016/j.mad.2009.08.003. PubMed PMID: 19698733. Pubmed Central PMCID.

[30] Martorana A, Balistreri CR, Bulati M, Buffa S, Azzarello DM, Camarda C, et al. Double negative (CD19+IgG+IgD-CD27-) B lymphocytes: a new insight from telomerase in healthy elderly, in centenarian offspring and in Alzheimer's disease patients. Immunol Lett 2014;162(1 Pt B):303–9. https://doi.org/10.1016/j.imlet.2014.06.003. PubMed PMID: 24951896. Pubmed Central PMCID.

[31] Bulati M, Buffa S, Candore G, Caruso C, Dunn-Walters DK, Pellicano M, et al. B cells and immunosenescence: a focus on IgG+IgD-CD27- (DN) B cells in aged humans. Ageing Res Rev 2011;10(2):274–84. https://doi.org/10.1016/j.arr.2010.12.002. PubMed PMID: 21185406. Pubmed Central PMCID.

[32] Hara Y, Tashiro Y, Murakami A, Nishimura M, Shimizu T, Kubo M, et al. High affinity IgM(+) memory B cells are generated through a germinal center-dependent pathway. Mol Immunol 2015;68(2 Pt C):617–27. https://doi.org/10.1016/j.molimm.2015.10.003. PubMed PMID: 26514429. Pubmed Central PMCID.

[33] Veneri D, Ortolani R, Franchini M, Tridente G, Pizzolo G, Vella A. Expression of CD27 and CD23 on peripheral blood B lymphocytes in humans of different ages. Blood Transfus 2009;7(1):29–34. https://doi.org/10.2450/2008.0007-08. PubMed PMID: 19290077. Pubmed Central PMCID: PMC26522.

[34] Seifert M, Kuppers R. Human memory B cells. Leukemia 2016 Dec;30 (12):2283–92. https://doi.org/10.1038/leu.2016.226. PubMed PMID: 27499139. Pubmed Central PMCID.

[35] Khaskhely N, Mosakowski J, Thompson RS, Khuder S, Smithson SL, Westerink MA. Phenotypic analysis of pneumococcal polysaccharide-specific B cells. J Immunol 2012;188(5):2455–63. https://doi.org/10.4049/jimmunol.1102809. PubMed PMID: 22271652. Pubmed Central PMCID: PMC3288481.

[36] Xiang FF, Zhu JM, Cao XS, Shen B, Zou JZ, Liu ZH, et al. Lymphocyte depletion and subset alteration correlate to renal function in chronic kidney disease patients. Ren Fail 2016;38(1):7–14. https://doi.org/10.3109/0886022X.2015.1106871. PubMed PMID: 26539739. Pubmed Central PMCID.

[37] Canada MCo. Clinical Laboratory Tests- Normal Values, White Blood Cells and Differentials [Available from: <https://mcc.ca/objectives/normal-values/>.

[38] Morbach H, Eichhorn EM, Liese JG, Girschick HJ. Reference values for B cell subpopulations from infancy to adulthood. Clin Exp Immunol 2010;162 (2):271–9. https://doi.org/10.1111/j.1365-2249.2010.04206.x. PubMed PMID: 20854328. Pubmed Central PMCID: PMC2996594..

[39] Qin L, Jing X, Qiu Z, Cao W, Jiao Y, Routy JP, et al. Aging of immune system: Immune signature from peripheral blood lymphocyte subsets in 1068 healthy adults. Aging (Albany NY) 2016;8(5):848–59. PubMed PMID: 26886066. Pubmed Central PMCID: PMC4931839. doi:10.18632/aging.100894.

[40] Pahl MV, Gollapudi S, Sepassi L, Gollapudi P, Elahimehr R, Vaziri ND. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. Nephrol Dial Transplant 2010;25(1):205–12. https://doi.org/10.1093/ndt/gfp397. PubMed PMID: 19684120. Pubmed Central PMCID: PMC2796898.

[41] Mahmoodi M, Aghamohammadi A, Rezaei N, Lessan-Pezeshki M, Pourmand G, Mohagheghi MA, et al. Antibody response to pneumococcal capsular polysaccharide vaccination in patients with chronic kidney disease. Eur Cytokine Netw 2009;20(2):69–74. https://doi.org/10.1684/ecn.2009.0153. PubMed PMID: 19541592. Pubmed Central PMCID.

[42] Grzegorzewska AE. Hepatitis B vaccination in chronic kidney disease patients: a call for novel vaccines. Expert Rev Vaccines 2014;13(11):1317–26. https://doi.org/10.1586/14760584.2014.944508. PubMed PMID: 25148051. Pubmed Central PMCID.

[43] Hawdon N, Nix EB, Tsang RS, Ferroni G, McCready WG, Ulanova M. Immune response to Haemophilus influenzae type b vaccination in patients with chronic renal failure. Clin Vaccine Immunol 2012;19(6):967–9. https://doi.org/10.1128/CVI.00101-12. PubMed PMID: 22539472. Pubmed Central PMCID: PMC3370432.