

Abstract

Introduction: The measurement of serotype specific pneumococcal antibody (Ab) concentration has become a standard part of the assessment of patients with recurrent infection. However, the determination of Ab concentration alone may not always provide sufficient information about the patient's functional Ab capacity. The use of a functional assessment parameter has been useful in several clinical trials designed to evaluate vaccine efficacy. One surrogate marker for Ab functionality is the avidity of the Ab response. The ability to develop higher avidity Ab is an indication of normal B cell function and correlates with optimal protection. The purpose of this study was to develop and standardize a quantitative method to measure avidity of specific Ab to pneumococcal serotypes.

Methods: The chaotropic dissociation method was used to measure the strength of the Ab-antigen interaction (i.e., avidity). With this method, increasing doses of the chaotropic agent sodium thiocyanate (NaSCN) are used to dissociate the Ab-antigen complex, with higher doses of NaSCN being required with the higher avidity Ab. The dose resulting in 50% dissociation was defined as the avidity index (AI). The patient sera were from patients being evaluated for recurrent infection. The testing platform was the microbead array instrument from Luminex, which is the method most commonly used to quantify the pneumococcal Ab concentration in clinical laboratories.

Results: The method was able to generate reproducible AI values with most CV% in the 5 to 20% range, depending on the Ab concentration and avidity. AI provided unique discrimination of sera that was independent of the Ab concentration. There was no correlation of the [Ab] and AI (R^2 value= 0.048). The pneumococcal Ab in sera from unimmunized individuals was generally low in both [Ab] and AI. However, the response to Pneumovax demonstrated all combinations of high or low [Ab] and AI (see representative data below).

Conclusions: The method is reproducible and produces unique information about the patient's anti-pneumococcal response. It may be a reasonable surrogate for B cell maturation and function and therefore may be useful in the evaluation of patients with recurrent infection.

Background

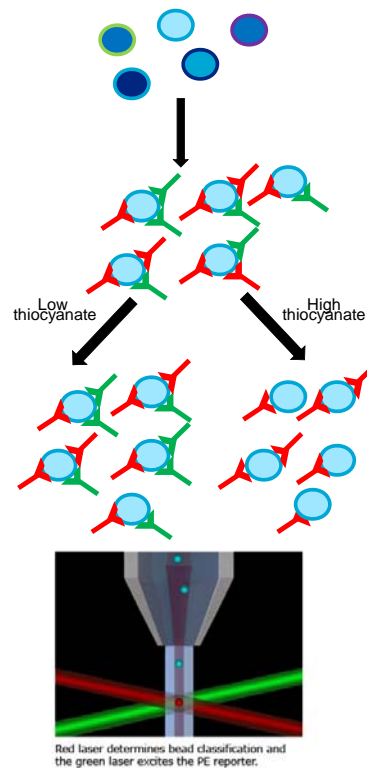
The evaluation of patients with recurrent infection is an important part of the practice of allergy-immunology today. In addition to the history and the clinical findings, laboratory tests are often useful. These tests include quantification of serum immunoglobulins, complement, T cell function, etc. In addition, the response to vaccine challenge is often helpful. In this regard, the measurement of serotype-specific pneumococcal antibody (Ab) concentration has become a standard part of the assessment of patients with recurrent infection. However, the interpretation of the pneumococcal antibody concentration measurements is not yet standardized. How many serotypes constitute an acceptable response (one or majority)? What criteria should be used to determine a good response (2 to 4 fold, minimum threshold, etc.)?

The determination of Ab concentration alone may not always provide complete information about the patient's functional Ab capacity. Are there more specific tests for the actual functional or protective capacity of the Ab produced? Can we determine if B cell memory and maturation is normal in the patient? One surrogate marker for Ab functionality is the avidity of the specific Ab response. The ability to develop higher avidity Ab is an indication of normal B cell function and correlates with optimal protection. It has been reported that avidity functions as an important determinant of antipneumococcal antibody protective efficacy against pneumococci (Usinger and Lucas) and a good correlation between avidity and opsonophagocytic activity has also been reported (Musher et al.). The purpose of this study was to develop and standardize a quantitative method to measure the avidity of serotype specific Ab to pneumococcal serotypes.

Materials and Methods

Quantitation and Avidity Evaluation of Pneumococcal antibodies

Avidity Determination. The term for the strength of binding between an antibody and antigen (Ag) is the avidity (or functional affinity). It is a complex thermodynamic parameter reflecting the association constant of the binding site, the valence of the Ab and the Ag, heterogeneity of the Ab, etc. The chaotropic dissociation method was used to measure the strength of this Ab-antigen interaction. With this method, increasing doses of the chaotropic agent sodium thiocyanate (NaSCN) are used to dissociate the Ab-antigen complex, with higher doses of NaSCN being required with the higher avidity Ab (Pullen et al). The dose resulting in 50% dissociation was defined as the avidity index (AI).



Pneumococcal polysaccharides are coupled to microspheres (each serotype is bound to a unique fluorescent microsphere)

Anti-pneumococcal Ab in the serum bind to Pne-PS coated microspheres

NaSCN is added to the microspheres (higher avidity antibodies (red) will require more thiocyanate to break the antigen/antibody interaction)

The microspheres are passed through a flow cell in the Bio-Plex™ reader. The reader generates a mean fluorescence intensity value (MFI) for each bead that is proportional to the amount of PE conjugate bound to the bead.

Figure 1. Measurements of Antibody binding by Multiplex Analysis

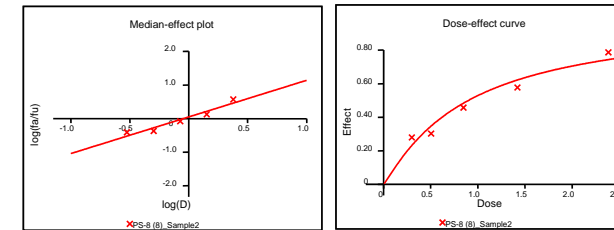
Calculation. The median effect equation was used to determine the average avidity of the Ab for each specific capsular serotype. A plot of log of fa/fo (fraction affected or dissociated/fraction unaffected) versus the log of the chaotropic agent (NaSCN) was analyzed with the CalcuSyn software (Chou 2006 and Chou and Martin) and the dose at the 50% dissociation point is determined. The slope of the median-effect plot line indicates the shape of the dose-response curve. The r value indicates the fit of the data to the median-effect plot; a minimum r value of 0.9500 was required for data to be accepted. The antilog of the x-intercept of the plot gives the median effect (50% inhibition) dose. All data points are equally weighted, without bias for those nearest the 50% inhibition dose.

Patient Serum Samples

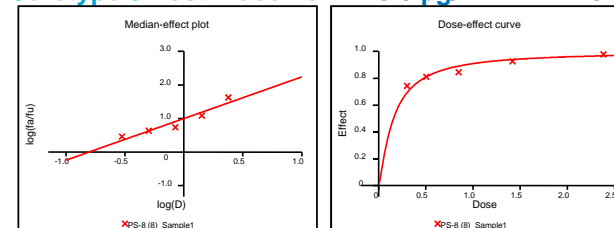
The sera used in these studies were discarded, de-identified sera from patients who had previously been tested for anti-pneumococcal antibody responses. In most cases, both pre- and post-vaccination sera were tested.

Results

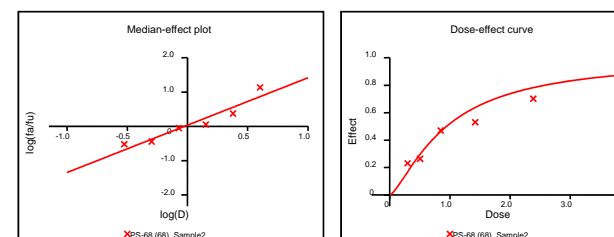
A Serotype 8 Pre- Vaccine 0.3 µg/mL AI=0.9



B Serotype 8 Post- Vaccine 19.5 µg/mL AI= <0.3



C Serotype 68 Pre- Vaccine 0.5 µg/mL AI= 0.94



D Serotype 68 Post- Vaccine 19.1 µg/mL AI= >4.0

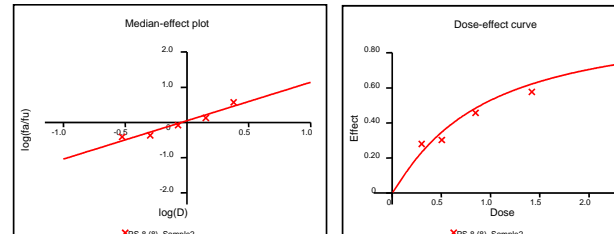


Figure 2. Antibody avidity is determined by the amount of SCN that decreases binding by 50%. The plots of log of fa/fo versus the log of the chaotropic agent are shown on the left and the dose vs. effect on the right. Pre-vaccine antibody levels can be of both high and low avidity. Vaccination can result in the expansion of both low (B) and high (D) avidity antibodies

Table I. Inter-Assay Precision for the Pneumococcal Avidity assay. Precision across 13 serotypes from 3 samples resulted in an average percent CV of 12.2.

| Serotype | Day 1 | Day 2 | Day 3 | % CV |
|----------|-------|-------|-------|------|
| 9 | 1.7 | 1.4 | 1.6 | 9.8 |
| 12 | 0.5 | 0.4 | 0.5 | 6.8 |
| 14 | 1.0 | 0.9 | 1.1 | 8.4 |

Results

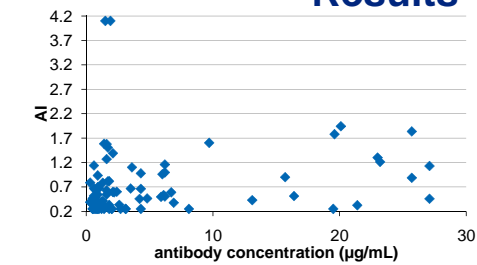


Figure 3. Antibody avidity is independent of antibody concentration. Post-vaccine serum samples (n=8) were tested for concentration and avidity against 12 different serotypes.

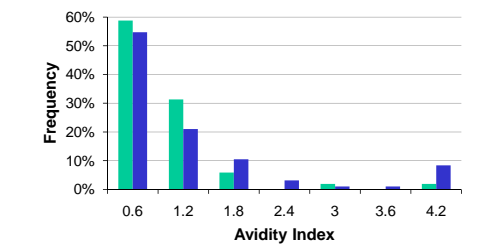


Figure 4. Antibody avidity distribution often shifts from pre-vaccine (green bars) to post-vaccine (blue bars) samples. Data were pooled from 8 patient sera.

Table 2. Pre- and Post-vaccine avidity in two representative samples, ND indicates not determined due to low antibody levels. Sample 1 illustrates a sample where the avidity did not change following vaccination and sample 2 illustrates some increases and some decreases in avidity following vaccination.

| | PS 1 | PS 3 | PS 4 | PS 8 | PS 9 | PS 12 | PS 14 | PS 19 | PS 23 | PS 26 | PS 51 | PS 56 | PS 68 |
|----------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| #1- pre | 0.9 | 1.0 | 0.4 | 1.1 | 0.3 | ND | 0.6 | 1.1 | 0.5 | 1.3 | 1.2 | 1.4 | 2.8 |
| #1- post | 0.4 | 0.7 | 0.3 | 0.7 | <0.3 | 0.4 | 0.6 | 1.2 | 0.3 | 1.0 | 0.9 | 1.9 | 4.2 |
| #2- pre | 1.07 | <0.3 | ND | 0.90 | ND | <0.3 | 0.91 | 0.57 | ND | ND | 0.89 | 1.15 | 0.94 |
| #2- post | 1.15 | 0.35 | <0.3 | <0.3 | 1.13 | <0.3 | 2.88 | 0.51 | 0.55 | <0.3 | >4.0 | 1.60 | >4.0 |

Conclusions

- The chaotropic agent NaSCN can be used to determine the avidity of multiple serotype-specific antibody specificities.
- The resulting Avidity Index is independent of Ab concentration.
- The Avidity Index (AI) provides unique information on B cell maturation and antibody function.
- The measurement of the AI may be useful in the evaluation of patients with recurrent infection. Prospective clinical studies are underway to determine the utility of AI, AI in combination with Ab concentration, or Ab concentration alone in the evaluation of patient responses.

References

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