Author’s response to reviews

Title: Clinical Implications and Characterization of Group A Streptococcus Infections in Adults with Cystic Fibrosis

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For detailed response see the supplemental material...

Response to comments from the reviewers.

Below we have included the comments as we received them from each of the two reviewers in black. We have gone to great lengths to adjust our manuscript to try and address each of these comments. These generally clarify and streamline the paper making for a better overall manuscript. In almost every instance, we have chosen to follow with the advice of the reviewer and incorporated their suggested changes into the manuscript. Our clarification to each of these comments can be found immediately following each comment in blue. We have included
comments in this fashion for transparency. In addition to the adjusted manuscript we have included, we have also included a “track changes” version as well. Thank you for the opportunity to address each of these.

Reviewer #1: BMC Pulmonology 2015-60

General comment

This manuscript describes a retrospective study examining the role of group A streptococcus (GAS) in pulmonary exacerbation in adult CF patients. Not surprisingly, GAS is found quite infrequently in adult CF patients with the organism detected in 15 patients in a study period of 37 years. Further there is no evidence that this organism is involved in the chronic infectious process that occurs in CF patients. However there is a bit of data suggesting that it rarely is involved in pulmonary exacerbations in a quite small subset of CF patients. The data is mainly negative.

Specific comments

1. It is appears from table 2 that in only 4 patients (and 5 episodes) was pulmonary exacerbation associated with GAS since the other 3 patients had other organisms well described in the literature to cause CF pulmonary exacerbation. Of the 4 GAS only patients, 2 had what was characterized as severe exacerbation, the definition of which was hospitalization and IV antimicrobial. Since these patients were not evaluated for the presence of viruses such as influenza, it is unclear with the severity of illness in these two was due to GAS although given the well-recognized pathogenicity of GAS, it is quite feasible that it alone could be responsible for exacerbation. The authors should comment in their discussion about the possibility at least of severe exacerbation associated with GAS could be secondary to viral agents which were not sought in this study.

We have revised the manuscript to soften the language and emphasize the observed association. We have not inferred causality. It now reads:

• “However, the presence of GAS increased the risk of PEx relative to the preceding clinic visit, particularly if present as the numerically dominant sputum pathogen. This finding may warrant treating individuals with GAS in their sputum with anti-GAS treatments in order to potentially avoid an ensuing PEx. However, other
factors including exacerbation of chronically infecting pathogens, and inter-current upper respiratory viral illnesses could also have contributed (although these factors were just as likely in comparator clinical visits). “

2. The authors' discussion of ceftazidime activity against GAS on pg 3 of the discussion Ln 24-26 is pure nonsense and should be eliminated. First ceftazidime is not used to treat GAS ever, secondly the breakpoints for ceftriaxone and ceftazidime are different so they can not be compared by showing extremely large zone series for ceftazidime and "sensitive" for ceftriaxone. This is the kind "data" presentation that lacks rigor and scientific validity and should be avoided.

The reviewers point is well taken. However, the message we intend to convey is to a treating clinician – who must provide treatment prior to sputum culture results. We have tried to convey the point that a common antimicrobial used empirically in CF, ceftazidime, does not have the same potential anti-GAS activity as other commonly antibiotics that might be used. We would suggest clinicians treating an exacerbation with ceftazidime where GAS is isolated look to change therapies.

We have revised the manuscript. It now reads in two sections (results, discussion);

• “CLSI breakpoints for GAS do not exist, however ceftazidime (an agent commonly used in CF) demonstrated considerably inferior anti-GAS activity (median KB zone of 33 mm).”

• “Importantly, our GAS strains were sensitive to antibiotics commonly used in the empiric treatment of CF PEx, although ceftazidime, an antibiotic commonly used in the empiric management of PEx, demonstrated considerably less activity.”

3. The authors try to "fluff up" their paper by characterizing the isolates for quorum sensing molecules, different enzymes and PFGE. Molecular typing is better accomplished by emm typing rather than PFGE. Why clinicians reading this paper would care about quorum sensing and production of different enzymes in this organism especially when there are no clinical correlates stated is unclear.
Stating we are “fluffing up” this manuscript is incorrect. CF clinicians have a long and storied history of recognizing the importance of individual virulence factors on disease pathogenesis. We have adapted the following information to rationalize our approach. The following detailed sections have been added.

• “Within CF, it is clear that mere culture status may not convey the entire story. Indeed, differential pathogenic potential has been observed with the expression of a number of phenotypic traits of classical CF pathogens including P. aeruginosa, Bcc, and S. aureus. For example, compared to patients with chronic methicillin-sensitive S. aureus (MSSA) infection, those with chronic methicillin-resistant S. aureus (MRSA) have an increased risk of death(1). Patients with MRSA have increased rates of lung function decline (2) and are even less likely to recover lung function following PEx (3). In patients with chronic P. aeruginosa infections, its conversion to a hyper-alginate producing, mucoid phenotype is associated with progressive decline in lung function, increased risk of hospitalization and reduced survival (4-8). The opposite appears true in Bcc chronically infected patients where mucoidy appears protective and patients with non-mucoid isolates experience an exaggerated rate of clinical decline (9,10). Even the ability to persist within the CF lung seems to be influenced by specific phenotypic traits of P. aeruginosa causing initial infections (11). The phenotypes that are associated with these strains may themselves not be directly involved in disproportionate lung disease, but rather they may be an indirect marker. Accordingly, we sought to characterize easily assayable and important virulence traits within infecting GAS strains to determine if these factors disproportionally modified PEx risk.”

PFGE is an alternate means of typing relative to emm typing – one which correlates very well. We utilized PFGE owing to our own expertise, its low cost and abundant supporting evidence in the literature. We have added to the manuscript;

• PFGE has been shown to be similarly effective at differentiating commonly infecting clones of GAS as other established typing modalities including emm gene typing. Using PFGE we demonstrated strain persistence in those patients with repeated positive cultures, rather than repeated new infections with different strains. We did
identify two patients with the same GAS isolate by PFGE, but propose this was unlikely to be patient-patient spread. Whereas typical CF pathogens are rare and opportunistic of the general population, GAS commonly colonizes the upper respiratory tract in the general population and common strains persist in locals for extended period of times. Furthermore, GAS from these patients were identified more than 2.5 years apart with multiple negative cultures in the ensuing time period making CF patient-patient transmission biologically implausible.”

4. In Materials and method: What is a “Wallac Victor2 and who manufactures it? This has been adjusted in the manuscript.

5. In phenotypic and genotypic characterization of GAS: GAS carriage is well described in the literature. The more cogent point might be that GAS carriage was infrequent in this population rather than that it occurred. Since there is so little data about carriage presented, perhaps nothing at all should be said about it.

Whereas persistent carriage in the UPPER AIRWAYS of the general population have been well identified, no data on persistent carriage in the LOWER AIRWAYS have ever been presented. These two factors are indeed very different. Our manuscript has been revised to read;

- “Using PFGE we demonstrated strain persistence in those patients with repeated positive cultures, rather than repeated new infections with different strains. We did identify two patients with the same GAS isolate by PFGE, but propose this was unlikely to be patient-patient spread. Whereas typical CF pathogens are rare and opportunistic of the general population, GAS commonly colonizes the upper respiratory tract in the general population and common strains persist in locals for extended period of times. Furthermore, GAS from these patients were identified > 2.5 years apart with multiple negative cultures in the ensuing time period making CF patient-patient transmission biologically implausible.”
6. Para 1 discussion: When the number of deaths are stated, it is likely reference 23 is an incorrect citation. Should it not be reference 11 here as well?

We have adjusted the manuscript accordingly. Mortality data was obtained from Carapetis et al and Public Health Agency of Canada. The mortality statistics from the Public Health Agency of Canada were omitted from the manuscript as the source did not provide a direct reference. Lionel et al did not include specific mortality statistics.

7. Para 2 discussion: Most reference material do not list GAS as a common cause of CAP. It would seem that 11% of CAP is due to GAS is very much an overestimation. Frankly I can not remember the last time we had an autopsy culture of the lungs positive for GAS and if the mortality is 20 to 38% than we should see it perhaps as often as once or twice a year. This entire paragraph is an overstatement.

High quality population outcomes based data exist for GAS invasive infections. Our data on case fatality was taken from the following citations.


• “The case fatality rate was 38% for GAS pneumonia”


• “Case-fatality ratios for pneumonia, necrotising fasciitis, and central nervous system infections exceeded 20% “

These were cited in the manuscript and we have not adjusted the text accordingly.
The prevalence quoted for GAS CAP was corrected in manuscript

8. Discussion para 3: "at one point 5% of patients isolated GAS. This seems improbable given that looking at table 3 the most isolates I see in a single year is 3. Does that mean you have only 60 patients in your cohort? Perhaps this statement should be re-thought.

The manuscript has been clarified and now reads;

1. “Between 1978 and 2013, there were fifteen individuals from a cohort of 318 adults with CF (4.7%) who had GAS isolated from their sputum.”

Reviewer #2: The high degree of complexity of the CF lung flora is now well recognised although the clinical significance of most taxa present is not yet understood. These authors have focussed their attention on Streptococcus pyogenes (the group A Streptococcus - GAS), a significant pathogen and potential cause of community acquired pneumonia, in order to determine the incidence, natural history and clinical impact of GAS infections in CF and to characterize the isolates both phenotypically and genotypically. The authors have at their disposal the considerable resource of the Southern Alberta Adult CF Clinic Biobank which allows retrospective evaluation of longitudinal microbiological and clinical data on adult CF patients between 1978 and 2013 and to further examine GAS isolates that had been obtained.

This study determines that GAS infection in CF lower airways is relatively rare (15/318 individuals = 4.7%) with the majority (13/15) having single isolations (transient GAS infection) that do not impact lung function, long term disease status nor disease progress. Despite the low numbers of GAS positive patients the authors were able to conclude that identification of GAS in CF spuotas correlated with pulmonary exacerbation (PEx), especially when the GAS were numerically dominant. The latter finding was highlighted as potentially informing treatment strategies taking GAS into account in order to reduce the risk of PEx. No association between expression of specific virulence factors and PEx were determined.

Although many of the findings here are negative I am of the opinion that this paper contributes to the increasing understanding of the complex variations and dynamics inherent to CF lung infections. Members of this group have previously published seminal papers that focus on the complexity of the polymicrobial nature of the infected CF lung. In contrast this paper does not (and presumably cannot) include much in the way of microbiological context; on page 14 line 9 they state that risk of PEx was not affected by the presence of specific chronic cultured
microorganisms - can this be expanded to describe exactly what is meant here? I would like to see some qualitative comment at least on the possibility (probability) of other members of the flora impinging on these data.

The manuscript has been adjusted. It now reads.

“This finding may warrant treating individuals with GAS in their sputum with anti-GAS treatments in order to potentially avoid an ensuing PEx. Certainly, other factors including exacerbation of chronically infecting pathogens, and inter-current upper respiratory viral illnesses could also have contributed although these factors were just as likely in comparator clinical visits. “

On page 15 line 44 the authors state that there was no association between production of particular virulence factors and the occurrence of PEx at isolation (data not shown) - in the abstract they state that GAS isolates produced variable levels of protease...etc; the data are shown in the Table as 'yes' or 'No' for expression - how is this 'variable levels' and was there any variation in the zone sizes between isolates? I think this should be made clearer.

The text has been adjusted to clarify how individual virulence factors were measured. It now reads;

“Production of virulence factors was scored as positive (production of the factor) or negative (no evidence of this particular factor). “