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Analysis of Autoantibody Profiles in Two Asbestiform Fiber Exposure Cohorts

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Abstract

An increased risk for Systemic Autoimmune Diseases (SAID) was reported in the population of Libby, Montana, where extensive exposure to asbestiform amphiboles occurred through mining and use of asbestiform fiber-laden vermiculite. High frequencies of antinuclear autoantibodies (ANA) were detected in individuals and mice exposed to Libby Asbestiform Amphiboles (LAA). Among the 6603 individuals who have undergone health screening at the Center for Asbestos Related Diseases (CARD, Libby MT), the frequencies of rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, and systemic sclerosis are significantly higher than expected prevalence in the United States. While these data support the hypothesis that LAA can trigger autoimmune responses, evidence suggests that chrysotile asbestos does not. Serological testing was therefore performed in subjects exposed to LAA or predominantly chrysotile (New York steamfitters) using multiplexed array technologies. Analyses were performed in order to determine a) autoantibody profiles in each cohort, and b) whether the two populations could be distinguished through predictive modeling. Analysis using perMANOVA testing confirmed a significant difference between autoantibody profiles suggesting differential pathways leading to autoantibody formation. ANA were more frequent in the LAA cohort. Specific autoantibodies more highly expressed with LAA-exposure were to histone, ribosomal P protein, Sm/Ribonucleoproteins, and Jo-1 (histidyl tRNA synthetase). Myositis autoantibodies more highly expressed in the LAA cohort were Jo-1, PM100, NXP2, and Mi2a. Predictive modeling demonstrated that anti-histone antibodies were most predictive for LAA exposure, and anti-Sm was predictive for the steamfitters' exposure. This emphasizes the need to consider fiber types when evaluating risk of SAID with asbestos exposure.

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Disclosure of Interest:

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Keywords

asbestos; autoantibodies; Libby Montana; chrysotile; amphibole

Introduction

The history of epidemiological studies exploring an association between asbestos exposure and autoantibody responses has recently been reviewed (Pfau et al. 2014). Since the mid-20th century, cross-sectional investigations noted the following in association with asbestos exposures: B cell humoral responses, including rheumatoid factor (RF) and antinuclear autoantibodies (ANA), increased serum IgG/IgA, and circulating immune complexes (Pfau, et al. 2014). After it was revealed that the population of Libby, Montana, had been exposed to asbestiform amphibole fibers through mining and widespread use of asbestiform fiber-laden vermiculite, subjects exposed to Libby Asbestiform Amphiboles (LAA) were found to display elevated frequency and titers of ANA compared to an age- and sex-matched reference population (Pfau, et al. 2005). The most frequent autoantibodies detected in the serum of those subjects were against common systemic lupus erythematosus (SLE) autoantigens, including dsDNA, histone, SSA/Ro52, and ribonucleoproteins (RNP) (Pfau, et al. 2005; 2009). The material present in the Libby vermiculite was originally described as enriched in tremolite, a federally-regulated form of amphibole asbestos (Meeker, et al. 2003). However, subsequent analyses revealed that the bulk of the material consists of "unregulated" asbestiform amphibole fibers, drawing questions as to whether it could be called "asbestos" (Boettcher 1967). In this study, the term LAA is used for the exposure from the Libby vermiculite, since clearly LAA is a collection asbestiform fibers and produces asbestos-related diseases (Cyphert, et al, 2016; Kodavanti et al, 2014; Larson, et al. 2012, Sullivan 2007, Whitehouse, et al. 2008).

Despite the presence of autoantibodies in asbestos-exposed cohorts, even in the context of current suggested criteria for environmental exposures, epidemiological evidence in support of an association between asbestos exposure and systemic autoimmune disease (SAID) is not robust (Noonan and Pfau 2011, Pfau, et al. 2014, Miller, et al. 2012b). Rheumatoid arthritis (RA) is the SAID most frequently associated with asbestos exposure (Greaves 1979, Noonan, et al. 2006, Olsson, et al. 2004), and an increased frequency of deaths attributed to systemic sclerosis (SSc) was reported for a cohort with likely occupational asbestos exposure (Gold, et al. 2007). In 2006, a case-control study of self-reported SLE patients, nested within a medically screened general population cohort in Libby, MT, showed a more than 4-fold elevated risk for SLE associated with a history of greater LAA exposure via multiple environmental pathways (Noonan, et al. 2006). Among the 6603 individuals who have undergone health screening at the Center for Asbestos Related Diseases (CARD, Libby MT), the frequencies of RA, SLE, sarcoidosis, and SSc are significantly higher than the published expected prevalence for these diseases in the United States (Diegel, et al. 2018). However, unlike investigations of crystalline silica (Cooper, et al. 2010, Pollard 2016), there are no apparent large cohort analyses of risk for SAID that establish asbestos or asbestiform structures as a trigger for autoimmunity. When examining the literature, there was a consistent lack of precision regarding the types of asbestos to which subjects were exposed,

such that it was often not possible to determine whether exposures were to amphibole or chrysotile asbestos. However, there appeared to be stronger evidence for autoimmune responses associated with amphibole asbestos (Pfau, et al. 2014). Subsequently, animal model studies compared ANA frequencies in mice exposed to LAA compared to chrysotile, and found that, while LAA led to significantly elevated frequencies of positive ANA tests compared to control mice, chrysotile did not (Ferro, et al. 2013, Zebedeo, et al. 2014). Further, a subset of steamfitters from New York were tested for ANA who displayed a very low frequency of positive ANA findings compared to the Libby cohort, accompanied by no reported cases of SLE, RA or SSc (Pfau, et al. 2015). Steamfitters are exposed to asbestos through their work with materials such as gaskets, valves and pumps associated with the pipes that they service and repair. While such asbestos-containing materials are not manufactured in the United States, they have been imported for many years, such that these occupations remain among the top of those reporting asbestosis (Walters, et al. 2018). For steamfitters, working with pipe gaskets is a major source of their exposure to asbestos (Longo, et al. 2002). Several publications report that gaskets contain almost entirely chrysotile asbestos, up to 80% by weight, with no amphibole reported (McKinnery, et al. 1992; Millette, et al. 1995; Longo, et al. 2002). Amphibole asbestos exposures may have occurred due to crocidolite or amosite-containing materials used by these workers, but chrysotile appears to have been predominant (Longo, et al. 2002).

The current study was performed to test the hypothesis that a mixture of fibers containing predominantly chrysotile affects B cell autoantibody response differently than asbestiform amphibole, LAA specifically. The objectives were to determine whether (1) the autoantibody profiles expressed in the LAA cohort were significantly different from the Steamfitters cohort, and (2) whether a particular set of autoantibodies was predictive for exposure to LAA.

Materials & Methods

Recruitment

Recruitment for LAA-exposed subjects was performed through the Libby Epidemiology Research Program (LERP, ATSDR 2008–2015) under approved Institutional Review Board protocols, and restricted to current or former residents of the cities of Libby and Troy, Montana. The LAA cohort is a cross-sectional population of the individuals who were screened and/or treated for asbestos-related diseases through the Center for Asbestos Related Diseases (CARD) in Libby, Montana, with no additional inclusion/exclusion criteria.

All subjects were over 18 years of age, with no restrictions regarding sex, smoking history, work history, or medical diagnosis. The cohort includes subjects who worked at the mine or associated occupations, as well as people who simply resided in the area. Due to the pervasive presence of the asbestiform amphiboles in the soil, tree bark, home insulation, worker clothing brought home, gardens, playgrounds, ball parks, gravel roads and ambient dust, all residents had at least some exposure. Work and residential histories obtained during screening were analyzed using a matrix developed specifically for the Libby exposures for which data regarding fiber concentrations are not generally available (Noonan, et al. 2015). Recruitment of subjects included notices in local newspapers and word-of-mouth, in

addition to recruitment through the health screening program at the CARD in Libby. All subjects completed extensive medical history questionnaires and provided a blood sample for serum collection by standardized operating protocols. The entire research protocol of the LERP was approved by the Icahn School of Medicine Institutional Review Board and was conducted in compliance with The Code of Ethics of the World Medical Association (Helsinki Declaration) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

The Steamfitters cohort consists of members of the steamfitters union in the state of New York who participated in health screening through the Icahn School of Medicine at Mt Sinai, New York City. Work histories obtained during the screening were analyzed using a scoring matrix developed by the Icahn School of Medicine at Mt Sinai, including frequency of various job activities that resulted in exposure to asbestos, and years of work. The scoring matrix was developed based on the clinical screening recommendations of experts in the field (Levin, et al. 2000). This cohort was also part of the LERP study described above, with research approved under the same IRB.

The following were exclusion criteria for subject serum samples to be used in the analyses: a) known lupus-inducing prescription medications, b) hemolyzed serum sample. The total number of serum samples tested was 484, with 397 from the LAA cohort, and 87 from the Steamfitters cohort.

Autoantibody Testing—Aliquots of sera were stored at −80C until required for serology assays. Autoantibody testing was performed at Mitogen Advanced Diagnostics Laboratory in Calgary, Alberta, Canada. The extractable nuclear antibody (ENA) profile utilized an addressable laser bead immunoassay (ALBIA) provided by TheraDiag (FIDIS: Paris, France). Autoantibodies to myositis-associated targets were detected by a line immunoassay (LIA) provided by Euroimmun (EUROLINE Autoimmune Myopathy Profile: Lübeck, Germany). Both ALBIA and LIA followed the protocols of the respective manufacturers. Target antigens in the ALBIA ENA profile included dsDNA, Sm, ribonucleoprotien (RNP), ribosomal P protein, proliferating cell nuclear antigen (PCNA), SSA/Ro60, SSB/La, Ro52/TRIM21, Scl-70 (topoisomerase I); and in the myositis LIA included Jo-1 (histidyl tRNA synthetase), Mi-2, Mi2α, MDA5, NXP2, TIF1γ, PL7, PL12, PM/Scl, Ku, SRP, EJ, OJ). In addition, antibodies to chromatin were detected by an enzyme-linked immunoassay (ELISA: Inova Diagnostics Inc., San Diego, CA). Cutoffs were established by the use of internal calibrators provided by the manufacturers and control sera included with each assay run. Results were expressed as optical density (OD) for LIA results, and median fluorescence units (MFU) or chemiluminescence intensity units (CIU) for ALBIA.

Antibodies to rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) were previously evaluated, and neither cohort had more than 3–4 subjects positive for these autoantibodies which was not statistically elevated above control populations (Pfau et al. 2005; Pfau et al. 2009; Pfau et al. 2014). These antibodies were therefore not included in this analysis.

Data Analysis—All statistical analyses were performed using R version 3.3.2 (R Core Team 2016). The perMANOVA procedure (Anderson 2001) using Euclidean distance was

implemented on the log(x+1) antibodies to test for a global difference in cohort antibody profiles. As this test showed strong evidence of differences in antibody profiles between cohorts, individual antibodies were examined. As antibody concentrations were heavily right-skewed, the two-sample Rank-Sum test (also known as the Wilcoxon test and the Mann-Whitney test, (Hollander and Wolfe 1973)) was used to screen for differences in median antibody levels between cohorts and for differences between sexes in the LAA cohort. Cohort comparisons were also performed using a subset of the LAA cohort that had occupational exposure. Spearman correlation coefficients were used to test for associations between antibody levels and a) body-mass index (BMI), and b) exposure. Since the exposure measures were not comparable between cohorts due to different metrics of exposure assessment, these comparisons were done separately within each cohort. In the LAA cohort with available exposure data (N=268), total occupational exposure was compared with antibodies only in subjects that had occupational exposure present (N=189). Total exposure (occupational plus environmental) was compared with antibodies in all subjects. For the Steamfitters cohort with available exposure data (N=35), antibody levels were compared with years of occupation and a sum of work history indicators. All p-values were adjusted to account for multiple testing (Benjamini and Hochberg 1995). Antibodies with an adjusted p-value less than 0.05 were flagged.

Statistical Learning to Develop Diagnostic Classifier—Within each cohort, approximately 2/3 of the subjects were randomly selected to serve as a training dataset to develop a diagnostic classifier, with the remaining 1/3 left aside as a validation dataset to assess the classifier performance.

A random forest (Breiman 2001) was constructed using the randomForest R package (Liaw and Wiener 2002) to predict whether a subject was exposed to amphibole or chrysotile asbestos. Random Forests are a popular machine learning technique known for having excellent predictive ability, low computational time, and fewer tuning parameters compared to other sophisticated classification techniques. They are constructed by sequentially estimating classification trees (1,000 total in this instance) using bootstrapped samples from the original data. For each split in the tree, a random subset of predictors is selected (approximately the square-root of the number of predictors), and the best split among these candidates was performed. Predictions for new observations were based on a majority vote of the individual tree predictions. The performance of the final model was quantified using the Area Under the Receiver Operating Characteristic Curve (AUC) for the validation data using the pROC R package (Robin, et al. 2011).

Results

Demographics and Exposure

Table 1 provides the demographic information of the study cohorts. The LAA cohort included both males and females, with minor differences in the ages of males compared to females that were not practically meaningful (average difference of 2.7 years). The Steamfitters cohort was numerically younger than the LAA cohort, but by less than 4 years. The Steamfitters cohort was all male. Additional analyses were performed to demonstrate

that the inclusion of women in the LAA cohort did not affect the outcomes: When examining the difference in the median antibody level between the two cohorts, all but one antibody (PCNA) had similar magnitude and sign of the estimated difference in the "men only" version of the data compared to the full version of the data. For the adjusted p-values, the same differences were detected with the same strength of evidence when using the CARD "men only" group compared to the full cohort. After accounting for multiple comparisons, no differences in median antibody level were detected between sexes in the LAA cohort. Lastly, for the predictive modeling, the "men only" group predicted equally well, with the same distinguishing patterns in the ANA antibodies.

Table 1 also gives the percent of each study group that tested positive during ANA screening, and the frequency of physician-diagnosed SAID reported in questionnaires. Using the serum dilution (1:80) and specific test used in this study (indirect immunofluorescence), background or normal populations in this age range (mean ~ 60 years) in the United States have a frequency of positive ANA tests around 20% (Satoh, et al. 2013), which is consistent with the Steamfitters cohort data in Table 1. In the same publication, ANA frequency peaked in the 50–59 year age group, with no further increase with increasing age. Therefore, the slightly lower average age in the Steamfitter cohort likely had no effect on the results. The only case of SAID in the Steamfitters cohort was sarcoidosis.

Data regarding latency between start of work and the current study are not available for many of the subjects. However, based on average ages at the time of blood work being 60 years old for both cohorts, and assuming a work history starting in the workers' 20's or 30's, the average latency for both cohorts is at least 30 years. In fact, for the Steamfitter cohort, the average work history was 31.3 years (standard deviation = 10.5).

Information on BMI is also presented in Table 1. There is no statistical difference in the mean BMI of the two cohorts (Table 1), and no associations between antibody levels and BMI were detected in either cohort (data not shown).

Table 2 presents data on exposure metrics for each of the cohorts. The exposure matrix available for the LAA cohort was developed for the LERP (Noonan et al. 2015), and the exposure matrix for the Steamfitters was developed by the Icahn School of Medicine at Mt Sinai. Both are based on self reported jobs and activities, and the frequencies of those jobs or activities. Because the matrices are different, the values for exposure in the two cohorts are not comparable. To provide some context for these exposures, the ranges reported in fibers/cc (by phase-contrast microscopy, PCM) for prevalent jobs in these cohorts are shown in the table (Noonan et al. 2015, Longo et al. 2002). Analyses were performed within each cohort to evaluate the effect of exposure level (matrix values) on the presence or types of antibodies. No associations between antibodies and exposure measures were detected in either cohort (data not shown).

Distribution of Panel of Autoantibodies in the Cohorts—Figure 1 illustrates the distribution of the different antibodies between the two cohorts. Each plot contains 2 box-plots (for the two cohorts) along with individual jittered points for each observation. The Y-axis shows the concentration units, representing OD, MFU, CIU, dependent on the assay for

the antibody, and illustrating relative serum antibody quantities for each subject. The first 13 plots are from the ENA panel (dsDNA through SSB), and the last 15 plots are from the myositis antibody panel. The last plot shows the distribution for anti-chromatin antibodies by ELISA.

Testing For Differences in Antibody Profiles Between Cohorts—Convincing evidence of a global difference in autoantibody profiles between cohorts was found (based on 14,999 permutations of cohort labels). Since a cohort difference in overall profiles was detected, the two-sample Rank-Sum test was used to test for a difference in median antibody levels between cohorts. Figure 2 is a graphical display of the strength of evidence (p-values) from the tests for each antibody. The $-\log_{10}$ (p-value) is displayed on the y-axis and the antibodies are on the x-axis. The solid horizontal line represents a negative $\log_{10}$ of 0.05, indicating that all autoantibodies with a point above this line had a raw p-value less than 0.05. The dashed horizontal line represents a negative $\log_{10}$ of 0.01, indicating that all autoantibodies with a point above this line had a raw p-value of less than 0.01. The red points indicate autoantibodies with an adjusted p-value less than 0.05. Similar differences were detected in the comparison when only using subjects from the LAA cohort that had occupational exposure (data not shown).

Classification of Cohorts Based on Autoantibodies—Data were used to develop a classification model that predicts whether a sample (based on the ENA antibodies) comes from the LAA cohort or from the Steamfitters cohort. This model achieved a validation AUC of 0.967, indicating an excellent ability to differentiate the cohorts using ENA autoantibody profiles. Figure 3 shows a parallel coordinates plot (PCP) for the 13 ENA antibodies that distinguish the two cohorts. The plot displays individual patients divided into LAA group (thin light-blue lines) and Steamfitters group (thin orange lines). A group average is shown as a thick blue line for LAA and a thick red line for Steamfitters. From this graph, differing shapes and heights of the peaks illustrate differences in the autoantibody expression profiles, with visually different peaks for antibodies to Sm, histone, Sm-RNP, ribosomes. Statistically, the model indicated that higher values for anti-histone antibodies were associated with a higher probability of being from the LAA cohort, while high values for Sm were associated with higher probabilities of coming from the Steamfitters cohort (Figure 3). However, it is important to note that only three people in the Steamfitters group, and 5 subjects in the Libby group, exhibited values for Sm autoantibodies that reached the respective cut-off for a positive test in the clinic. None of the Sm-positive Steamfitters subjects had a diagnosis of SAID, specifically SLE, while three of the Sm-positive subjects from Libby had a diagnosis of SAID. Data indicate that although assay values for antibodies to Sm were higher among the Steamfitters cohort than the LAA cohort, this was not necessarily indicative of clinically evident SLE.

Discussion

The results support and extend our previous studies, which reported elevated frequencies and titers of ANA in the Libby population, as well as an increased risk for SLE, SSc, RA, and sarcoidosis (Noonan, et al. 2006, Pfau, et al. 2005, Diegel, et al. 2018). Exposure to LAA was demonstrated to be associated with an elevated frequency of positive ANA tests in non-

autoimmune prone mice and rats as well (Ferro, et al. 2013, Pfau, et al. 2008, Salazar, et al. 2012). Mice exposed to tremolite were found to exhibit immune complex deposition in the kidneys and mild glomerular changes suggestive of SLE nephritis (Pfau, et al. 2008). Amosite, an amphibole asbestos, was also shown to induce ANA in rats (Salazar, et al. 2012). Chrysotile asbestos, however, did not elicit an ANA response above control levels in mice (Ferro, et al. 2013, Zebedeo, et al. 2014).

Predictive modeling demonstrated that antibodies to histone were most predictive for exposure to LAA, with high values of anti-histone predicting the LAA cohort with a high probability. Notably, Sm-RNP and PM-Scl antibodies were also predictive for the LAA cohort. Anti-histone antibodies are common in SLE, and are often associated with a syndrome called “drug-induced lupus” (DIL) which occurs in patients taking certain medications, such as procainamide or hydralazine, but which disappears after discontinuing the drug (Patel and Richardson 2013; Rubin 2015)). Interestingly, the epitopes targeted by anti-histone antibodies in DIL tend to vary from those targeted in SLE (Portanova, et al. 1987), possibly due to different types of post-translational modifications, such as acetylation, that create antigenic sites. In contrast, idiopathic SLE, histone-positive patients usually express multiple autoantibodies such as dsDNA, SSA/Ro60, Ro52, Ribosome, and Sm/RNP. It is important to point out that with the more recent use of biological therapeutics, the clinical and serological spectrum of drug-induced lupus has changed, where anti-dsDNA tends to be a dominant feature (Olsen 2004). In both of the cohorts in the present study, unlike DIL where anti-histone antibodies tend to occur in isolation, the anti-histone positive patients in our study tended to express multiple autoantibodies (including PCNA, Ro52, PM/Scl and Ribosome), and none were taking drugs associated with DIL, making DIL less likely. It would be interesting to determine the specific epitopes of these patients’ anti-histone antibodies to determine whether B cell specificity varies between the two cohorts, which suggest different triggers and pathways leading to the anti-histone response.

In this study, higher titers of anti-Sm were most predictive for exposure to predominantly chrysotile in the Steamfitters group. Anti-Sm is regarded as a highly specific biomarker for SLE, and its presence is a key serological component in diagnosis and classification criteria for that disease (Petri, et al. 2012). It is interesting that anti-Sm was a “predictive” antibody for the Steamfitters group in the current study, when Pfau, et al (2015) previously demonstrated that chrysotile was not associated with ANA autoantibodies in mice (Ferro, et al. 2013), and SAID is not frequent in the Steamfitters group (Table 1). Antigenic moieties on the Sm protein tend to be post-translational modifications such as dimethyl-arginine and/or have modifications that are associated with oxidative or apoptotic processes (Mahler 2011, Mahler et al. 2005), both of which occur with asbestos exposures (Blake, et al. 2007, Hamilton et al. 1996). It was not possible to distinguish specific peptide epitopes in the current study, which may be of value in future experiments due to evidence that antibodies to specific epitopes are more highly associated with disease (Mahler et al. 2005; Mahler 2011). Because none of the anti-Sm-positive patients in the Steamfitters group exhibited a clinically evident autoimmune disease, it is possible that the epitopes targeted by the anti-Sm antibodies in this group are different and associated with a benign pathogenic course compared to the anti-Sm induced by LAA. Further, as attested by diagnostic and classification criteria for the full spectrum of SAID, autoantibodies alone are not diagnostic

for disease. This is probably particularly true for autoantibodies that have no apparent pathological functions, such as anti-Sm, which are referred to as ‘indifferent’ or bear no known role in the pathogenesis of SAID (Fritzler 2012).

Autoimmune diseases are reported to be the consequence of an environmental trigger factor in the context of genetic predisposition (Bernatsky, et al. 2017b, Lee and Lawrence, 2018; Cooper, et al. 2010). In this setting, both the genetic and trigger components could have variability that leads to varying degrees of severity (Lee and Lawrence, 2018). Autoantibodies can occur during pre-clinical phases of disease development, with ultimate disease development being dependent on other variables (Choi, et al. 2016). With forms of asbestos, silica and nanofibers which all have complex surface properties, such variables might include cooperative cell interactions such as scavenger receptors which modulate responses in the early cellular responses including inflammasome activation, anti-oxidant regulation, and oxidative damage (Murthy, et al. 2015; Nakayama 2018; Pfau, et al. 2012; Thompson, et al. 2014). Further research is needed to determine specific variables relating to differential B cell responses.

Individually, many autoantibodies may be present at low levels, fluctuating in response to various environmental and endogenous stimuli. The ability of these two mixtures of fibers to stimulate significantly different profiles of autoantibodies suggests differences in their ability to elicit long-lasting and pathogenic effects on pathways activating antibody-producing B cells. The fact that significantly different cytokine profiles were reported in mice exposed to amphibole versus chrysotile fibers (Ferro, et al. 2013) further supports this premise. Amphibole asbestos is known to be highly inflammatory, driving chronic pleural inflammation, which has been reported to be less so with chrysotile exposure (Bernstein, et al. 2011; 2013). This inflammation is suggested as a trigger that affects the antibody profile, and may exacerbate the response to elicit clinical disease. More research is needed to test these hypotheses.

In order to support the hypothesis that the differences in the autoantibodies was associated with fiber type, several other variables were considered, including dose, BMI, and sex. When analyzed by exposure metrics, there were no changes in the overall results, suggesting that the autoantibody differences between the cohorts are not due to exposure “dose” differences. Further, when the Steamfitters cohort was compared to a subset of the LAA cohort with occupational exposures, the differences between antibodies remained the same as when the entire cohort was used.

Mean difference in BMI was insignificant between the cohorts, but there was a difference in the male: female ratios. Analyses were performed on the data that excluded any impact of sex or BMI on the differences in autoantibodies. Research suggests that BMI is not associated with ANA in otherwise healthy people, or has a negative association with ANA frequency decreasing as BMI increases (Satoh et al. 2012). Another variable that may have impacted the data is smoking history, which was missing for the Steamfitter cohort. The literature is somewhat mixed regarding any impact of smoking on SAID or autoantibody development. Most studies suggest that smoking may impact the presence or absence of anti-cyclic citrullinated peptide antibodies (CCP) (Pfau 2012), but not generalized ANA

(Satoh et al. 2012). Because anti-CCP antibodies were not previously detected above normal frequencies in either cohort, this antibody was not included in the analyses presented here. However, since it has recently been suggested that anti-CCP can be detected before clinical disease, particularly associated with exposure to pollution (Bernatsky, et al. 2017a), it would be of interest to explore this further, to evaluate even low levels of anti-CCP. Analysis of anti-CCP antibodies is planned in addition to further exploration of the specific histone and Sm epitopes being recognized by autoantibodies in these cohorts.

The importance of this study is two-fold. First, it is an approach to fulfilling criteria needed for proposing that LAA, like crystalline silica, is an environmental trigger for systemic autoimmune disease by providing comparative data with another exposure (Miller, et al. 2012b). Multiple large epidemiological studies were needed to make the case that crystalline silica is a trigger for SAID (Miller, et al. 2012a), and there simply are no large cohorts for amphibole asbestos exposures in order to conduct similar experiments for amphibole asbestos, with the possible exception of the Wittenoom crocidolite exposure cohorts in Western Australia (Berry, et al. 2012) where current studies are underway (Reid, et al 2018). There are currently few occupational exposure cohorts, and more environmental asbestos exposures, with the latter being more difficult to track subjects or assess exposure. More creative approaches are needed, and thus a statistical model was constructed to assess the ability of autoantibodies to predict exposure to LAA compared to predominantly chrysotile, and therefore to distinguish various types of asbestos exposure. It is essential to make that distinction due to the many differences being described in the health outcomes for LAA compared to chrysotile asbestos (Bernstein et al. 2005, Cyphert, et al. 2012;2016, Ferro, et al. 2013, Li et al. 2012). Second, it sets the stage to begin studies that assess the pathogenic determinants of LAA that increase the risk for autoantibody production and for autoimmune diseases, and to evaluate the clinical rheumatic outcomes of exposure to different asbestiform fibers.

Conclusions

The data from this study supported the hypothesis that there is a detectable difference between the two exposure cohorts in terms of the overall frequency of autoantibodies and the specific autoantibodies expressed. LAA induced a set of autoantibodies that frequently occur in SLE patients, including antibodies to dsDNA, histone, ribosomal P protein, Sm/RNP, Scl-70 (topoisomerase I), PM/Scl, and SSA/Ro60. The Steamfitter cohort also expressed some autoantibodies, although to a lesser degree, including Sm, PM/Scl, Ro52, SSA/Ro60 and SSB. Both cohorts showed expression at low frequencies of myositis antibodies, including PM75, SAE1, and NXP2, but higher expression was particularly evident in the LAA cohort for OJ, NXP2, and Mi2a. These differences emphasize the need to consider fiber types when evaluating risk of autoimmune outcomes attributed to asbestos exposure. It further provides data to enable careful evaluation of the ability of different mineral fibers to initiate modification, through oxidative or apoptotic processes, of target epitopes that lead to immune responses to the modified peptides. Finally, it supports the hypothesis that subsets of autoantibodies are predictive of exposure to LAA, thus providing a tool for future evaluation of the association between amphibole asbestos and autoimmune disease.

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References

Anderson MJ 2001 A new method for non-arametric multivariate analysis of ariance. Austral Ecol 26: 32–46. doi:Doi 10.1046/J.1442-9993.2001.01070.X

Benjamini Y, and Hochberg Y. 1995 Controlling the false discovery rate: A practical and powerful approach to multiple testing. J Roy Stat Soc Ser B 57289–300.

Bernatsky SM, Smargiassi A, Joseph L, Awadall P, Comegna I, Hudson M, Fritzler MJ. 2017 Industrial air emissions, and proximity to major industrial emitters, are associated with anti-citrullinated protein antibodies. Environ Res 157:60–63. [PubMed: 28525857]

Bernatsky SM, Pfau JC, and Fritzler MJ. 2017 Environmental exposures and biomarkers predictive of rheumatoid arthritis and the pathway to precision medicine. J Lab Precision Med 2: 1–4.

Bernstein D, Dunnigan J, Hesterberg T, Brown R, Velasco JA, Barrera R, Hoskins J, and Gibbs A. 2013 Health risk of chrysotile revisited. Crit Rev Toxicol 43: 154–83. doi: 10.3109/10408444.2012.756454 [PubMed: 23346982]

Bernstein DM, Chevalier J, and Smith P. 2005 Comparison of Calidria chrysotile asbestos to pure tremolite: Final results of the inhalation biopersistence and histopathology examination following short-term exposure. Inhal Toxicol 17: 427–49. doi:10.1080/08958370591002012 [PubMed: 16020039]

Bernstein DM, Rogers RA, Sepulveda R, Donaldson K, Schuler D, Gaering S, Kunzendorf P, Chevalier J, and Holm SE. 2011 Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite-asbestos following short-term inhalation exposure. Inhal Toxicol 23: 372–391. doi:10.3109/08958378.2011.575413 [PubMed: 21639707]

Berry G, Reid A, Aboagye-Sarfo P, de Klerk NH, Olsen NJ, Merler E, Franklin P, and Musk AW. 2012 Malignant mesotheliomas in former miners and millers of crocidolite at Wittenoom (Western Australia) after more than 50 years follow-up. Br J Cancer 106: 1016–1020. doi:10.1038/bjc.2012.23 [PubMed: 22315054]

Blake DJ, Bolin CM, Cox DP, Cardozo-Pelaez F, and Pfau JC. 2007 Internalization of Libby Amphibole Asbestos and induction of oxidative stress in murine macrophages. Toxicol Sci 99: 277–288. doi:10.1093/toxsci/kfm166 [PubMed: 17578862]

Boettcher AL 1967 The Rainy Creek alkaline-ultramafic igneous complex near Libby, Montana. J Geol 75526–53.

Breiman L 2001 Random forests. Machine Learning 45(1): 5–32. doi: 10.1023/A1010933404324

Choi MY, Barber MR, Barber CE, Clarke AE, and Fritzler MJ. 2016 Preventing the development of SLE: Identifying risk factors and proposing pathways for clinical care. Lupus 25: 838–849. doi: 10.1177/0961203316640367 [PubMed: 27252260]

Cooper GS, Wither J, Bernatsky S, Claudio JO, Clarke A, Rioux JD, Ca NGI, and Fortin PR. 2010 Occupational and environmental exposures and risk of systemic lupus erythematosus: silica, sunlight, solvents. Rheumatology 49: 2172–2180. doi:10.1093/rheumatology/keq214 [PubMed: 20675707]

Cyphert JM, Nyska A, Mahoney RK, Schladweiler MC, Kodavanti UP, and Gavett SH. 2012 Sumas Mountain chrysotile induces greater lung fibrosis in Fischer344 rats than Libby Amphibole, El

Dorado tremolite, and Ontario ferroactinolite. Toxicol Sci 130: 405–415. doi:10.1093/toxsci/kfs249 [PubMed: 22903825]

Cyphert JM, McGee MA, Nyska A, Schladweiler MC, Kodavanti UP, Gavett SH. 2016 J Toxicol Environ Health A 79: 49–60. [PubMed: 26818398]

Diegel R, Black B, Pfau JC, McNew T, Noonan CW, Flores R. 2018 Case series: rhematological manifestations attributed to exposure to Libby asbestiform amphiboles. J Toxicol Environ Health A, 81: 734–747. [PubMed: 29927712]

Ferro A, Zebedeo CN, Davis C, Ng KW, and Pfau JC. 2013 Amphibole, but not chrysotile, asbestos induces anti-nuclear autoantibodies and Il-17 in C57bl/6 mice. J Immunotoxicol 11: 283–290. doi:10.3109/1547691X.2013.847510 [PubMed: 24164284]

Fritzler MJ 2012 Toward a new autoantibody diagnostic orthodoxy: understanding the bad, good and indifferent. Auto Immun Highlights 3: 51–58. doi:10.1007/s13317-012-0030-7 [PubMed: 26000127]

Gold LS, Ward MH, Dosemeci M, and De Roos AJ. 2007 Systemic autoimmune disease mortality and occupational exposures. Arthritis Rheum 56: 3189–3201. doi:10.1002/art.22880 [PubMed: 17907164]

Greaves IA 1979 Rheumatoid “pneumoconiosis” (Caplan’s Syndrome) in an asbestos worker: A 17 years’ follow-up. Thorax 34: 404–405. [PubMed: 483218]

Hamilton RF, Iyer LL, and Holian A. 1996 Asbestos induces apoptosis in human alveolar macrophages. Am J Physiol 271(5 Pt 1): L813–9. [PubMed: 8944725]

Hollander M, and Wolfe DA. Nonparametric statistical methods. New York: John Wiley & Sons, 1973.

Kodavanti UP, Andrews D, Schladweiler MC, Gavett SH, Dodd DE, Cyphert JM. 2014 Early and delayed effects of naturally occurring asbestos on serum biomarkers of inflammation and metabolism. J Toxicol Environ Health A 77: 1024–1039.. [PubMed: 25072823]

Larson TC, Lewin M, Gottschall EB, Antao VC, Kapil V, and Rose CS. 2012 Associations between radiographic findings and spirometry in a community exposed to Libby amphibole. Occup Environ Med 69: 361–366. doi:10.1136/oemed-2011-100316 [PubMed: 22383589]

Lee F and Lawrence DA. 2018 From infections to anthropogenic inflicted pathologies: Involvement of immune balance. J Toxicol Environ Health B 21: 24–46.

Levin SM, Kann PE, Lax MB. 2000 Medical examination for asbestos-related disease. Am J Indust Med 37:6–22.

Li M, Gunter ME, and Fukagawa NK. 2012 Differential activation of the inflammasome in Thp-1 Cells Exposed to Chrysotile Asbestos and Libby “Six-Mix” Amphiboles and subsequent activation of BEAS-2B cells. Cytokine 60: 718–730. doi:10.1016/j.cyto.2012.08.025 [PubMed: 23017228]

Liaw A, and Wiener M. 2002 Classification and regression by Randomforest. R News 2: 18–22.

Longo WE, Egeland WB, Hatfield RL, Newton LR. 2002 Fiber release during the removal of asbestos-containing gaskets: A work practice simulation. App. Occup Environ Hygiene 17:55–62.

Mahler M 2011 Sm peptides in differentiation of autoimmune diseases. Adv Clin Chem 54109–28.

Mahler M, Fritzler MJ, and Bluthner M. 2005 Identification of a Smd3 epitope with a single symmetrical dimethylation of an arginine residue as a specific target of a subpopulation of anti-Sm antibodies. Arthritis Res Ther 7: R19–R29. doi:10.1186/ar1455 [PubMed: 15642139]

McKinnery WN, Jr. and Moore RW. 1992 Evaluation of airborne asbestos fiber levels during removal and installation of valve gaskets and packing. Am Ind Hyg Assoc 53:531–532.

Meeker GP, Bern AM, Brownfield IK, Lowers HA, Sutley SJ, Hoefen TM, and Vance JS. 2003 The Composition and Morphology of Amphiboles from the Rainy Creek Complex, near Libby, Montana. Am Mineral 881955–69.

Miller FW, Alfredsson L, Costenbader KH, Kamen DL, Nelson LM, Norris JM, and De Roos AJ. 2012a Epidemiology of environmental exposures and human autoimmune diseases: findings from a National Institute of Environmental Health Sciences expert panel workshop. J Autoimmun 39: 259–271. doi:10.1016/j.jaut.2012.05.002 [PubMed: 22739348]

Miller FW, Pollard KM, Parks CG, Germolec DR, Leung PS, Selmi C, Humble MC, and Rose NR. 2012b Criteria for environmentally associated autoimmune diseases. J Autoimmun 39: 253–258. doi:10.1016/j.jaut.2012.05.001 [PubMed: 22771005]

Millette JR, Mount MD, Hays SM. 1995 Releasability of asbestos fibers from asbestos-containing gaskets. EIA Technical Journal, Fall1995: 10–15.

Murthy S, Larson-Casey JL, Ryan AJ, He C, Kobzik L, and Carter AB. 2015 Alternative activation of macrophages and pulmonary fibrosis are modulated by scavenger receptor, macrophage receptor with collagenous structure. FASEB J 29: 3527–3536. doi:10.1096/fj.15-271304 [PubMed: 25953850]

Nakayama M 2018 Macrophage recognition of crystals and nanoparticles. Front Immunol 9103. doi: 10.3389/fimmu.2018.00103

Noonan CW, Conway K, Landguth EL, McNew T, Linker L, Pfau J, Black B, Szeinuk J, and Flores R. 2015 Multiple pathway asbestos exposure assessment for a superfund community. J Expo Sci Environ Epidemiol 25(1): 18–25. doi:10.1038/jes.2014.25 [PubMed: 24756101]

Noonan CW, and Pfau JC 2011 Asbestos exposure and autoimmune disease In Encyclopedia of Environmental Health, edited by Nriagu J, : Elsevier, pp.193–203

Noonan CW, Pfau JC, Larson TC, and Spence MR. 2006 Nested case-control study of autoimmune disease in an asbestos-exposed population. Environ Health Perspect 114: 1243–1247. [PubMed: 16882533]

Olsen NJ 2004 Drug-induced autoimmunity. Best Pract Res Clin Rheumatol 18: 677–688. doi: 10.1016/j.berh.2004.05.006 [PubMed: 15454126]

Olsson AR, Skogh T, Axelson O, and Wingren G. 2004 Occupations and exposures in the work environment as determinants for rheumatoid arthritis. Occup Environ Med 61: 233–238. [PubMed: 14985518]

Patel D, and Richardson B. 2013 Drug-induced lupus: Etiology, pathogenesis, and clinical aspects In Dubois' Lupus Erythematosus and Related Syndromes, edited by Wallace DJ and Hahn BH, Philadelphia: Elsevier, pp.484–494

Petri M, Orbai AM, Alarcon GS, Gordon C, Merrill JT, Fortin PR, Bruce IN, Isenberg D, Wallace DJ, Nived O, Sturfelt G, Ramsey-Goldman R, Bae SC, Hanly JG, Sanchez-Guerrero J, Clarke A, Aranow C, Manzi S, Urowitz M, Gladman D, Kalunian K, Costner M, Werth VP, Zoma A, Bernatsky S, Ruiz-Irastorza G, Khamashta MA, Jacobsen S, Buyon JP, Maddison P, Dooley MA, van Vollenhoven RF, Ginzler E, Stoll T, Peschken C, Jorizzo JL, Callen JP, Lim SS, Fessler BJ, Inanc M, Kamen DL, Rahman A, Steinsson K, Franks AG, Jr., Sigler L, Hameed S, Fang H, Pham N, Brey R, Weisman MH, McGwin G, Jr., Magder LS. 2012 Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 64(8): 2677–86. doi:10.1002/art.34473. [PubMed: 22553077]

Pfau JC 2012 Rheumatoid arthritis In: Immunotoxicity, Immune Dysfunction, and Chronic Disease, Chapter 7. Edited by Luebke Robert and Dietert Rod. Humana Press, pp171–192.

Pfau JC, Blake DJ, and Fritzler MJ. 2009 Autoantibody profiles of an asbestos-exposed population" In Autoimmunity: Role, Regulation and Disorders, edited by Vogel FL and Zimmermann LF, New York, NY: Nova Science Publishers, pp245–268.

Pfau JC, Seib T, Overocker JJ, Roe J, and Ferro AS. 2012 Functional expression of system X(C)- Is upregulated by asbestos but not crystalline silica in murine macrophages. Inhal Toxicol 24: 476–485. doi:10.3109/08958378.2012.689782 [PubMed: 22697888]

Pfau JC, Sentissi JJ, Li S, Calderon-Garciduenas L, Brown JM, and Blake DJ. 2008 Asbestos-induced autoimmunity in C57bl/6 mice. J Immunotoxicol 5: 129–137. doi:10.1080/15476910802085756 [PubMed: 18569382]

Pfau JC, Sentissi JJ, Weller G, and Putnam EA. 2005 Assessment of autoimmune responses associated with asbestos exposure in Libby, Montana, USA. Environ Health Perspect 113: 25–30. [PubMed: 15626643]

Pfau JC, Serve KM, and Noonan CW. 2014 Autoimmunity and asbestos exposure. Autoimmune Dis 20141–11. doi:10.1155/2014/782045

Pfau JC, Serve KM, Woods L, and Noonan CW. 2015 Asbestos exposure and autoimmunity. In Otsuki Y Yoshioka A Holian (eds.) Biological effects of fibrous and particulate substances, 181–94. Current Topics in Environmental Health and Preventive Medicine: Springer Publications.

Pollard KM 2016 Silica, silicosis, and autoimmunity. Front Immunol 797. doi:10.3389/fimmu. 2016.00097

Portanova JP, Arndt RE, Tan EM, and Kotzin BL. 1987 Anti-histone antibodies in idiopathic and drug-induced lupus recognize distinct intrahistone regions. J Immunol 138: 446–451. [PubMed: 2432124]

R_Core_Team. "A Languange and environment for statistical computing." R Foundation for Statistical Computing, https://www.R-project.org/.

Reid A, Franklin P, de Klerk N, Creaney J, Brims F, Musk B, Pfau J. 2018 Autoimmune antibodies and asbestos exposure: evidence from Wittenoom, Western Australia. Am J Indust Med 61:615–620.

Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, and Muller M. 2011 Proc: An open-source package for R and S Plus to analyze and compare Roc curves. BMC Bioinformatics 12. doi:Artn 77 10.1186/1471-2105-12-77

Rubin RL 2015 Drug-induced lupus. Expert Opin Drug Saf 14: 361–378. doi: 10.1517/14740338.2015.995089 [PubMed: 25554102]

Salazar KD, Copeland CB, Wood CE, Schmid JE, and Luebke RW. 2012 Evaluation of anti-nuclear antibodies and kidney pathology in Lewis rats following exposure to Libby amphibole asbestos. J Immunotoxicol 10: 329–333. doi:10.3109/1547691X.2012.747230 [PubMed: 23256773]

Satoh M, Chan EKL, Ho LA, Rose KM, Parks CG, Cohn RD, Justko TA, Walker NJ, Germolec DR, Whitt IZ, Crockett PW, Pauley BZ, Chan JYF, Ross SJ, Birnbaum LS, Zeldin DC, Miller FW. 2012 Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 64:2319–2327 doi:10:1002/art.34380 [PubMed: 22237992]

Sullivan PA 2007 Vermiculite, respiratory disease, and asbestos exposure in Libby, Montana: Update of a cohort mortality study. Environ Health Persp 115: 579–585. doi:10.1289/ehp.9481

Thompson JK, Westbom CM, MacPherson MB, Mossman BT, Heintz NH, Spiess P, and Shukla A. 2014 Asbestos modulates thioredoxin-thioredoxin interacting protein interaction to regulate inflammasome activation. Part Fibre Toxicol 1124. doi:10.1186/1743-8977-11-24

Whitehouse AC, Black CB, Heppe MS, Ruckdeschel J, and Levin SM. 2008 Environmental exposure to Libby asbestos and mesotheliomas. Am J Ind Med 51: 877–880. doi:10.1002/ajim.20620 [PubMed: 18651576]

Zebedeo CN, Davis C, Pena C, Ng KW, and Pfau JC. 2014 Erionite induces production of autoantibodies and Il-17 in C57bl/6 mice. Toxicol Appl Pharmacol 275: 257–264. doi:10.1016/j.taap.2014.01.018 [PubMed: 24518925]

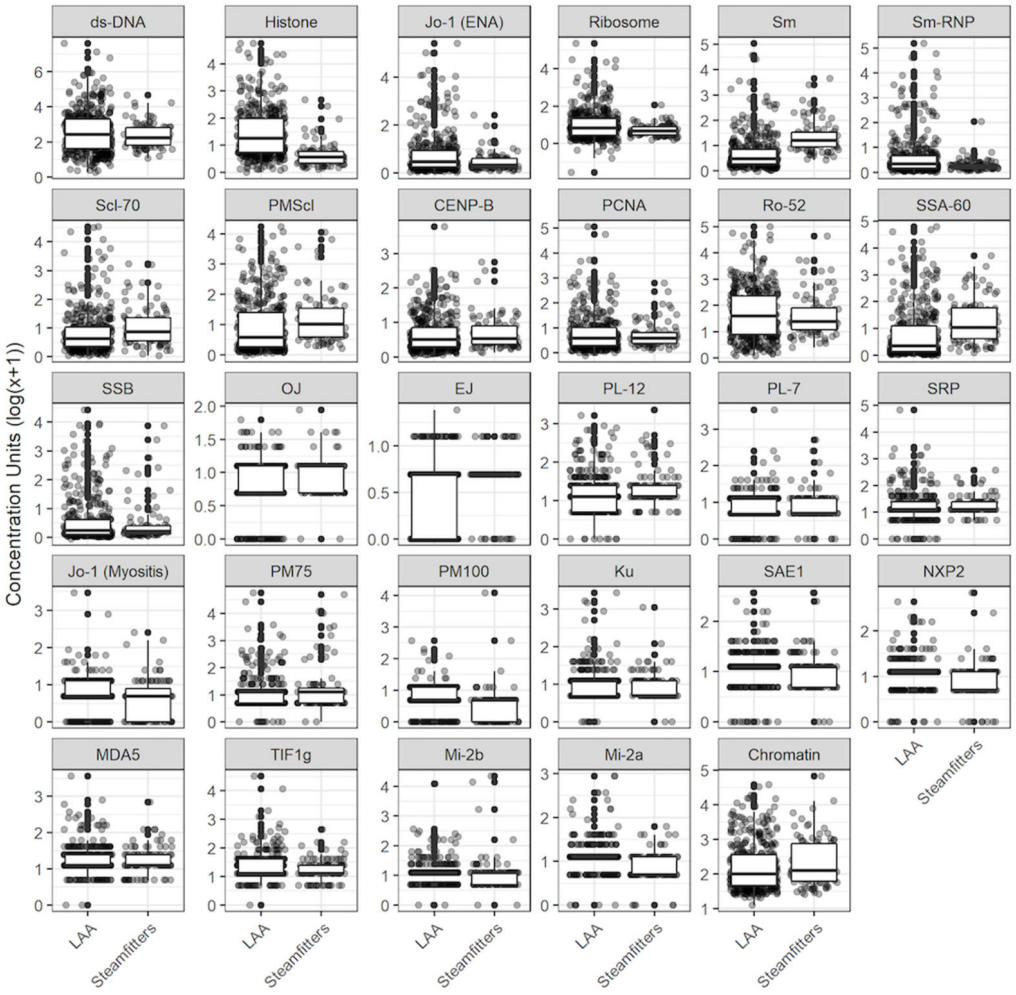

Figure 1:

Panel of plots examining the distribution of different antibodies (log(x+1) transformation) between the 2 cohorts. Each plot contains 2 box-plots (corresponding to the 2 cohorts, LAA on the left, Steamfitters right) along with individual jittered points along the x-axis for each of the individual observations.

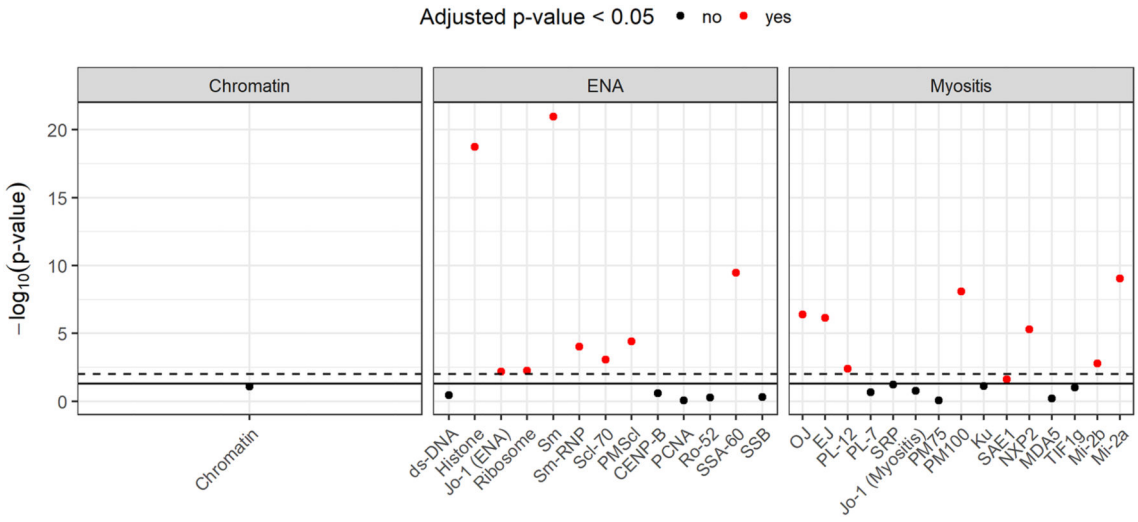

Figure 2:

Graphical display of the strength of evidence from individual Rank-Sum tests. The negative log (base 10) p-value (unadjusted) is displayed on the y-axis and the antibodies are displayed on the x-axis. The different panels represent the different groupings of the antibodies. The solid horizontal line represents a negative log (base 10) of 0.05, indicating that all antibodies with a point above this line had a raw p-value less than 0.05. The dashed horizontal line represents a negative log (base 10) of 0.01, indicating that all antibodies with a point above this line had a raw p-value less than 0.01 The red points indicate antibodies with an adjusted p-value less than 0.05.

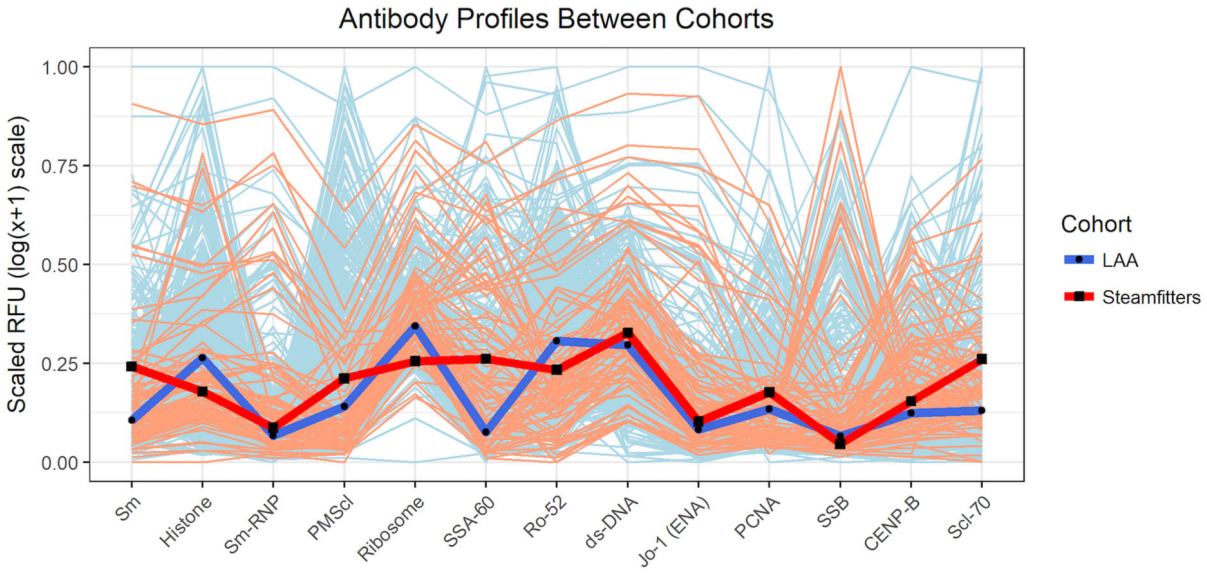

Figure 3:

A parallel coordinate plot (PCP) for the 13 ENA antibodies that distinguish LAA from Steamfitters. The plot displays individual patients from combined modeling (n=323) and validation cohort (n=161) divided into LAA group (thin light-blue lines) and Steamfitters group (thin orange lines). A group average is shown as thick blue line for LAA and thick red line for Steamfitters. The y-axis shows antibody measurements (log(x+1) transformation) scaled to 0–1 range.

Table 1

	LAA	Steamfitters	*P* value
N (females/males)	397 (158/239)	87 (0/87)	
Mean age (SD)	60.8 (11.7)	57.0 (9.1)	0.006[a]
Females, mean age (SD)	59.2 (11.8)	NA	
Males, mean age (SD)	61.8 (11.6)	57.0 (9.1)	<0.001[a]
Percent ANA Positive (HEp2)	43%	23%	0.001[b]
Males, Percent ANA Positive	42%	23%	0.01[b]
SAID[c] Diagnosis, # cases (%)	30 (7.6%)	1 (1.1%)	0.03[b]
Body-Mass Index (BMI), Mean (SD)	30.6 (7.3)	30.3 (4.9)	0.66[a]

a: Two-tailed, unpaired t-test

b: Fisher's Exact Test

c: RA, SLE, SSc, Sarcoidosis (One Steamfitter had Sarcoidosis)

Table 2

	LAA	Steamfitters
Environmt. exposure value, Mean (SD)	2.9 (4.5)[a]	NA
Occup. exposure value, Mean (SD)	9.3 (12.4)[a]	29.7 (16.4)[b]
Exposure Type		
Mostly Occupational	53.3%	100%
Mostly Environmental/Residential	46.7%	0%
Range reported for jobs in cohort (f/cc)	2.2-182.1[c]	1.2-144.2[d]
Tobacco use		No data
Current smoker	12.5%	
Former smoker	39.8%	
Never smoker	31.7%	

a: Using a scoring matrix developed for the Libby cohort (Noonan, et al. 2015)

b: Using a scoring matrix developed for the Mt Sinai School of Medicine

c: Noonan, et al. 2015

d: Longo, et al. 2002