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Serum Interleukin-6 Levels and Pulmonary Function in Ataxia-Telangiectasia

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Objective To evaluate the potential link between systemic inflammation and impaired lung function in people with ataxia-telangiectasia (A-T), we hypothesized that serum levels of interleukin (IL)-6, a proinflammatory cytokine, would correlate inversely with lung function in subjects with A-T.

Study design Consecutive subjects with A-T were recruited from the Johns Hopkins Outpatient A-T Clinical Center. Serum levels of IL-6 and 8 were measured by enzyme-linked immunosorbent assay. Spirometry was performed in subjects ≥6 years of age on the same day that serum was obtained for measurements of cytokines.

Results Approximately 80% of subjects had elevated serum IL-6 levels (>1.0 pg/mL). No association was found between elevated IL-6 and age. Elevated IL-8 levels were found in 23.6% of subjects, and all subjects with elevated IL-8 levels had elevated IL-6 levels. Subjects with elevated IL-6 levels (mean: 6.14 ± 7.47 pg/mL) had significantly lower mean percent forced vital capacity (FVC%, 50.5% ± 17.8%) compared with subjects with normal serum IL-6 levels (FVC% of 66.2 ± 16.1, $P = .018$). Greater IL-6 levels were associated with lower FVC% even after adjustment for receiving gamma globulin therapy ($P = .024$) and supplemental nutrition ($P = .055$).

Conclusions An association was found between elevated serum IL-6 levels and lower lung function in subjects with A-T. In addition, subjects with both elevated IL-6 and IL-8 had the lowest mean lung function. These findings indicate that markers for systemic inflammation may be useful in identifying individuals with A-T at increased risk for lower lung function and may help in assessing response to therapy. *(J Pediatr 2016;171:256-61)*.

The ataxia-telangiectasia mutated (ATM) pathway is involved intricately in the response to oxidative stress and injury repair. Individuals with ataxia-telangiectasia (A-T) have been shown to develop cerebellar degeneration, immunodeficiency, sensitivity to ionizing radiation, and increased risk of malignancies.[1-3] Sinopulmonary infections, impaired airway clearance, restrictive lung disease, and aspiration have been reported widely in children and adults with A-T.[4,5] In A-T, pulmonary symptoms often are not identified early in the disease process, and untreated lung disease can lead to significant morbidity and mortality. Therapies for the treatment of lung disease in A-T currently are supportive and include timely treatment of respiratory infections, pulmonary clearance techniques, and oral steroids for interstitial lung disease. Identifying risk factors and biomarkers associated with lung disease would allow for earlier treatment and improved outcomes.

Immune deficiency is present in more than 50% of people with A-T and can contribute to a decline in lung function.[6] Chronic interleukin (IL)-6 production, an inflammatory cytokine produced by monocytes, macrophages, and other cell lineages, has been found previously in diseases characterized by immune dysregulation.[7,8] In some of these diseases, IL-6 blockade has been shown to modify disease development and severity. In rheumatoid arthritis specifically, symptomatic improvement has been demonstrated in people who received a humanized anti-IL-6R monoclonal antibody. In A-T, the role of immune dysregulation and inflammation in disease presentation and progression currently is unclear.[9,10]

People with A-T have abnormal DNA damage responses, telomere shortening, and increased sensitivity to oxidative stress.[11,12] Oxidative stress and telomere shortening have been associated with inflammation-related diseases, including pulmonary fibrosis, neurodegeneration, and cancer,[13] conditions commonly seen in A-T. In an earlier retrospective study, we found that a subset of individuals with A-T had elevated levels of serum IL-8, a proinflammatory neutrophil chemoattractant.[14] This finding suggested a link between systemic inflammation and A-T. Both IL-6 and IL-8 have been shown to be elevated during conditions of nutritionally mediated oxidative stress and in stress-related inflammatory diseases.[15,16] Because IL-6 elevation has been reported in chronic diseases associated with immune dysregulation, we sought to determine in a prospective cross-sectional study whether serum IL-6 levels correlated with lung function in people with A-T.

A-T	Ataxia-telangiectasia
ATM	Ataxia-telangiectasia mutated
DDR	DNA damage repair
FEV1%	Percent Forced expiratory volume in 1 second
FVC%	Percent forced vital capacity
IL	Interleukin
IVIG	Intravenous immunoglobulin

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In this study we hypothesized that serum levels of IL-6 are associated inversely with lung function in people with A-T. To evaluate the potential association between lung function and IL-6 levels, we chose to limit spirometry data to that obtained on the same day that blood was drawn for cytokine measurement.

Methods

All subjects met the diagnosis of A-T on the basis of clinical symptoms and laboratory findings of either elevated alpha-fetoprotein, diminished ATM protein, and/or increased chromosomal breakage after in vitro exposure to x-rays as previously established.[17] Demographic information was obtained from chart review. The institutional review board of the Johns Hopkins Medical Institutions approved the study, and written informed consent was obtained from every participant and/or his/her guardian.

Venous blood was drawn from 61 individual subjects undergoing outpatient evaluation at the Johns Hopkins A-T clinic between 2012 and 2014. Three of the 61 subjects were seen twice during this time period, and the first clinic visit was used for analysis. Concentrations of IL-6 (n = 61) and IL-8 (n = 55) were determined by the use of commercially available EIA kits (R&D Systems, Minneapolis, Minnesota). These were quantitative sandwich enzyme immunoassays used according to the manufacturer's instructions. The optical density of each sample was determined with a microplate reader set to 450 nm (Optimax, Molecular Devices, Sunnyvale, California). Data were calculated from a standard curve and the results reported in picograms (pg) of cytokine protein per milliliter for each cytokine. The mean minimum detectable dose for IL-6 was 0.7 pg/mL with the Normal Adult Reference Range (Cytokine Laboratory, Johns Hopkins University, Baltimore, Maryland) between 0 and 1.0 pg/mL (n = 63). The mean minimum detectable dose for IL-8 was 3.5 pg/mL, with the Normal Adult Reference Range (R&D Systems, Minneapolis, Minnesota) being <31.2 pg/mL (n = 34). Samples were assayed in duplicate, and values were expressed as ±SD. All sample testing was performed in a masked setting.

Subjects who were 6 years of age or older and able to follow directions (n = 49) underwent standard spirometry according to recommendations by the American Thoracic Society[18] on the same day that serum was obtained for measurements of cytokines. During each visit, a minimum of 3 flow-volume curves were attempted per person (MedGraphics). The best flow volume loop per visit was selected for further evaluation. Wang-predicted values were used for children up to 16 years of age,[19] and the Third National Health and Nutrition Examination Survey–predicted values were used for adolescents older than 16 years of age.[20]

Statistical Analyses

Group comparisons and study-wide correlations were made by the use of nonparametric tests (Mann-Whitney *U*, Kruskal-Wallis, Fisher exact, Spearman correlation) because of the non-normal distribution of IL-6. When appropriate, multivariable linear regressions were performed with percent forced vital capacity (FVC%) as the dependent variable and the log of IL-6 levels and other covariates as the independent variables; co-efficient *P* values <.05 were considered evidence of association. Intercooled Stata 11 (StataCorp LP., College Station, Texas) was used for all statistical analyses.

Results

Demographic characteristics of subjects with A-T are summarized in Table I. Male and female subjects were represented equally. No differences among sexes were found with respect to either high (>1.0 pg/mL) or normal (≤1.0 pg/mL) serum IL-6. Subjects who had high serum IL-6 levels (mean: 6.14 ± 7.47 pg/mL) had a mean FVC% of 50.5 ± 17.8, and subjects with normal serum IL-6 levels (mean: 0.71 ± 0.13 pg/mL) had a mean FVC% of 66.2 ± 16.1 (*P* = .018).

Table I. Study demographics of subjects with serum IL-6 levels (n = 61)*

	Entire study population (n = 61)	Normal serum IL-6 level [0-1.0] (n = 12)	Elevated serum IL-6 level [>1.0] (n = 49)	*P* value
Sex (% male)	49.2%	58.3%	46.9%	.53
Age, y	13.5 ± 7.7 [1, 27]	11.8 ± 7.5 [2, 23]	13.9 ± 7.7 [1, 27]	.34
FVC%, predicted	53.7 ± 18.5 [17, 98] (n = 49)	66.2 ± 16.1 [44, 96] (n = 10)	50.5 ± 17.8 [17, 98] (n = 39)	.018
FEV1%, predicted	60.0 ± 19.7 [20, 107] (n = 49)	73.3 ± 15.8 [51, 99] (n = 10)	56.6 ± 19.4 [20, 107] (n = 39)	.010
Precancer/cancer (% yes)†	14.8%	0.0%	18.4%	.18
Cutaneous granulomas (% yes)	8.2%	8.3%	8.2%	1.00
Gamma globulin therapy (% yes)	31.2%	8.3%	36.7%	.08
Supplemental nutrition (% yes)‡	24.6%	8.3%	28.6%	.26
Serum IL-6, pg/mL	5.07 ± 7.03 [0.48, 36.26]	0.71 ± 0.13 [0.48, 0.97]	6.14 ± 7.47 [1.03, 36.26]	<.001
Serum IL-8, pg/mL	41.8 ± 72.0 [5.8, 467.8] (n = 55)	16.0 ± 5.3 [10.2, 30.2] (n = 12)	49.1 ± 80.0 [5.8, 467.8] (n = 43)	.042

*Values are mean ± SD [range] unless otherwise stated.
†Precancer conditions included myelodysplastic syndrome (n = 1).
‡Supplemental nutrition includes formula delivered by gastrostomy or jejunostomy feeding tubes and/or total parental nutrition delivered intravenously in the home setting.