

Letter to the Editor

Serum ferritin and transferrin levels are not serologic markers of toluene diisocyanate-induced occupational asthma

To the Editor:

In a recent article, Hur et al¹ reported that by using a proteomic approach, ferritin expression was downregulated whereas transferrin expression was upregulated in bronchoalveolar lavage fluid in subjects with methylene diphenyl diisocyanate-induced occupational asthma (MDI-OA) or eosinophilic bronchitis compared with exposed asymptomatic control workers (AECs) to methylene diphenyl diisocyanate (MDI). The authors also measured these compounds by ELISA using sera from the MDI-OA/eosinophilic bronchitis and AEC groups. The results showed that serum ferritin and transferrin can be serologic markers in diagnosing MDI-OA. To identify subjects with MDI-OA, the optimal serum cut-off levels were 69.84 ng/mL for ferritin and 2.48 mg/mL for transferrin. When these 2 parameters were combined, the sensitivity was 71.43%, and the specificity was 85.71%. These authors speculate that some susceptible subjects with defects in iron metabolism and lower serum ferritin levels may develop MDI-OA after MDI exposure, whereas some subjects with lower serum transferrin levels may be resistant to MDI exposure. The authors then argue that ferritin might act defensively against MDI, and some subjects with an impaired ferritin level may be more susceptible to MDI-OA than those who have a normal iron metabolism and are exposed to MDI.

This study was performed to confirm whether these serologic markers are associated with the phenotype of toluene diisocyanate occupational asthma (TDI-OA) and may therefore be used as serologic markers for the diagnosis of TDI-OA.

We enrolled in this study 17 subjects with TDI-OA, a mean age $\pm$ SD of 37.2 ± 11.6 years, and a median exposure to diisocyanates of 72 (range, 10-432) months; 12 asymptomatic exposed controls (AECs) with a mean age of 42.6 ± 14 years and a median of exposure to diisocyanates of 96 (range, 4-360) months; and 10 nonatopic healthy controls whose mean age was 33.3 ± 7.7 years.

Patients with Toluene diphenyl diisocyanate (TDI)-induced asthma had positive responses to the methacholine and TDI-specific inhalation challenge test. These tests were performed as previously described.²

AEC subjects were selected from among TDI-exposed workers in a single car upholstery factory. All subjects were screened by respiratory questionnaire, fraction of exhaled nitric oxide (NIOX MINO; Aerocrine, Solna, Sweden), and methacholine challenge test. All reported that they had experienced no work-related respiratory symptoms and while working had negative methacholine tests.

Healthy nonatopic volunteers were never exposed to TDI. Atopy was defined as more than 1 positive response to the common inhalant allergens on the skin prick test and included the common inhalant allergens.

Serum ferritin and transferrin in the subjects from the TDI-OA, AEC, and nonatopic healthy control groups were measured by using the Centaur platform (Siemens Medical Solutions, Germany). Serum levels of ferritin were retested using an ELISA kit (Alpha Diagnostic International, San Antonio, Tex). All tests were performed according to the manufacturer's instructions.

After applying normality tests (Shapiro-Wilk), log-transformed serum ferritin and transferrin values were compared by using ANOVA with Bonferroni correction for multiple testing. All values were also adjusted for age and sex by using the analysis of covariance (ANCOVA). Receiver operating characteristic curves were used to evaluate the validity of the serum ferritin and transferrin levels for discriminating between TDI-OA and AEC, and the area under the curve (AUC) with a 95% CI was computed. All computations were performed by using SPSS Version 17.0 (SPSS Inc, Chicago, Ill).

Serum ferritin and transferrin levels in the TDI-OA, AEC workers, and nonatopic healthy control groups are shown in Fig 1. There were no statistical differences in ferritin or transferrin values among the groups. These results were affected when adjusting for age and sex (mainly sex, ANCOVA; Table I). Exposure or time of exposure to diisocyanate did not influence ferritin or transferring values.

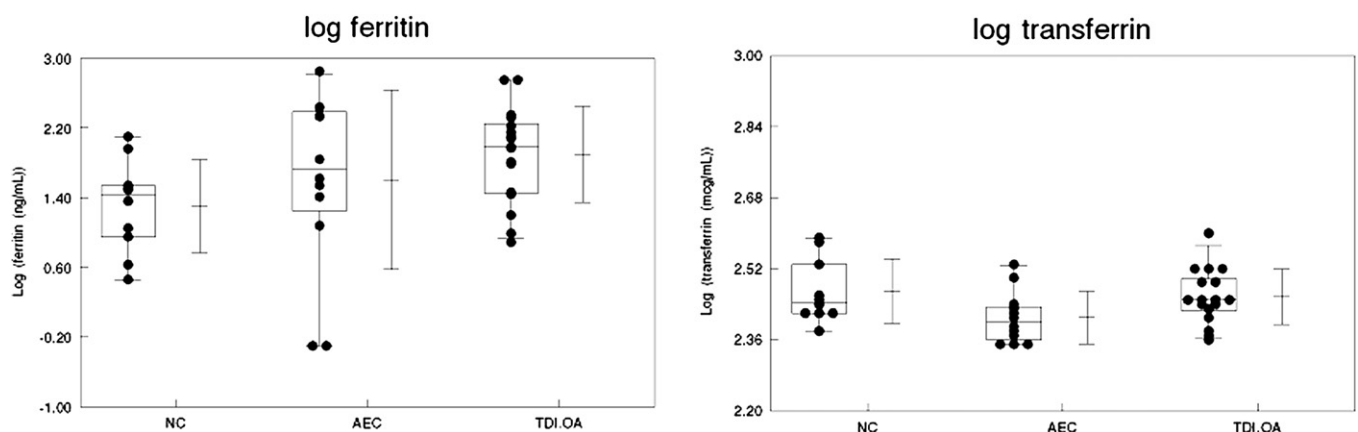


FIG 1. Ferritin and transferrin serum values in different groups. Lines in boxes indicate medians, and limits correspond to quantiles 1 and 3. Vertical lines indicate means and SDs.

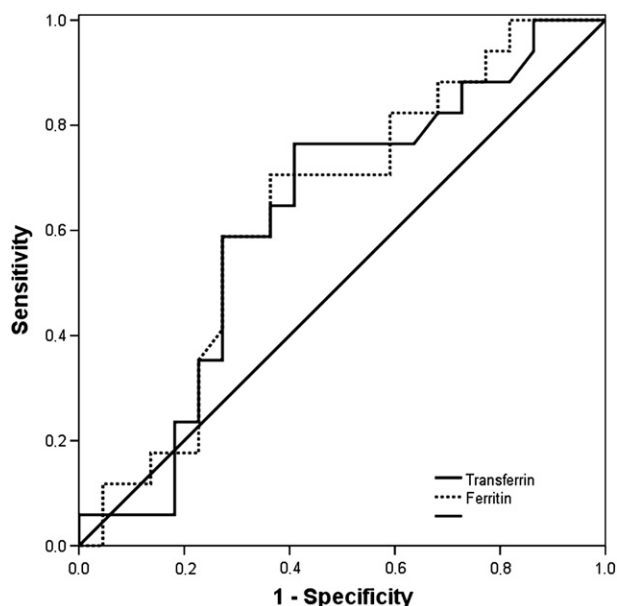
TABLE I. Comparisons of log ferritin and transferrin values between study groups

Log ferritin	P value	Log transferrin	P value
ANOVA*	.135	ANOVA*	.075
TDI-OA vs AEC	.893	TDI-OA vs AEC	.169
TDI-OA vs NC	.146	TDI-OA vs NC	1
AEC vs NC	1	AEC vs NC	.121
ANCOVA	<.001	ANCOVA	.038
TDI-OA vs AEC	.04	TDI-OA vs AEC	.25
TDI-OA vs NC	.259	TDI-OA vs NC	1
AEC vs NC	1	AEC vs NC	1

ANCOVA with Bonferroni correction to adjust for age and sex.

NC, Normal controls.

*ANOVA with Bonferroni correction.

**FIG 2.** AUC for ferritin and transferrin. Ferritin AUC = 0.638, 0.461-0.814 -CI 95%- $P = .14$; transferrin AUC = 0.636, 0.446-0.806 -CI 95%- $P = .18$.

Analysis of AUCs for ferritin or transferrin did not reach statistical significance when adjusting by age and/or sex (Fig 2). Therefore, no cut-off values can be obtained.

When applying recommended optimal cut-off values for transferrin and ferritin reported by Hur et al,¹ no significant association for diagnosis TDI-OA versus non-TDI-OA ($P = .46$ and $P = .11$, respectively; Fisher exact test) was found.

Our results did not confirm any of the aforementioned results published by Hur et al.¹ We did not find any statistical difference among the groups analyzed. In fact, ferritin levels were not down-regulated and transferrin levels were not upregulated in sera samples from the population we studied. However, we confirmed that ferritin and transferrin sera values were affected by age and sex. Neither can we confirm the cut-off values described in the previous study. We were unable to find a new cut-off values because

AUCs obtained did not reach statistical significance. The population included in our study is similar to the one included in Hur et al,¹ with the exception that our patients with occupational asthma had positive challenge test results to TDI, although some of them had been exposed to TDI and MDI and likely were sensitized to both diisocyanates. Clinical cross-reactivity between diisocyanates is common,³ although specific IgG responses to different diisocyanates have been described.⁴ Nevertheless, the pathogenesis of occupational asthma to different diisocyanates is considered to be similar for all of them,⁵ and our discrepant results cannot be explained by the fact that our subjects were sensitized to TDI.

In conclusion, in the population studied, serum ferritin and transferrin values did not differ among TDI-OA, TDI-exposed workers without occupational asthma, or healthy control patients, and therefore cannot be used as serologic markers for identifying TDI-OA. Further studies will be necessary to confirm whether these serologic markers are useful for the diagnosis of different types of diisocyanate-induced occupational asthma.

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