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Prolonged exposure to welding fumes as a novel cause of systemic iron overload

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Abstract

Background & Aims: Inhalation of welding fume may cause pulmonary disease known as welder's lung. At our centre we came across a number of welders with systemic iron overload and prolonged occupational history and we aimed at characterizing this novel clinical form of iron overload.

Methods: After exclusion of other known causes of iron overload, 20 welders were fully evaluated for working history, hepatic, metabolic and iron status. MRI iron assessment was performed in 19 patients and liver biopsy in 12. We included 40 HFE-HH patients and 24 healthy controls for comparison.

Results: 75% of patients showed lung HRCT alterations; 90% had s-FERR > 1000 ng/mL and 60% had TSAT > 45%. Liver iron overload was mild in 8 and moderate-severe in 12. The median iron removed was 7.8 g. Welders showed significantly lower TSAT and higher SIS and SIS/TIS ratio than HFE-HH patients. Serum hepcidin was significantly higher in welders than in HFE-HH patients and healthy controls. At liver biopsy, 50% showed liver fibrosis that was mild in four, and moderate-severe in two. Liver staging correlated with liver iron overload.

Conclusions: Welders with prolonged fume exposure can develop severe liver iron overload. The mechanism of liver iron accumulation is quite different to that of HFE-HH suggesting that reticuloendothelial cells may be the initial site of deposition. We recommend routine measurement of serum iron indices in welders to provide adequate diagnosis and therapy, and the inclusion of prolonged welding fume exposure in the list of acquired causes of hyperferritinemia and iron overload.

KEYWORDS

ferritin, hepcidin, iron overload, liver fibrosis, lung, welder

Abbreviations: BAL, Broncho Alveolar Lavage; ELISA, Enzyme-Linked ImmunoSorbent Assay; FEV1, Forced Expiratory Volume 1; FVC, Forced Vital Capacity; HIS, Hepatic Iron Stores; HRCT, High Resolution lung CT; IR, Iron Removed; LIC, Liver Iron Concentration; PIS, Portal Iron Score; PPD, Personal Protective Devices; s-FERR, serum-Ferritin; SIS, Sinusoidal Iron Score; TIS, Total Iron Score; TLCO, Diffusion Lung CO; TSAT, Transferrin saturation.

LAY SUMMARY

- The present study firstly demonstrates that a subgroup of welders with prolonged exposure to welding fume, and particularly those with less use of protective devices, can develop severe liver iron overload and liver damage.
- We recommend that measurement of serum iron indices should be part of the routine test in welders to identify subjects requiring more in-depth evaluation of iron overload and liver function, and to provide adequate measures for preventing and controlling occupational exposure to iron.

1 | INTRODUCTION

The experimental research and cases reported in the literature show that inhalation of welding fume for a prolonged period of time at high exposures may cause a form of pulmonary fibrosis similar to respiratory bronchiolitis, which in rare circumstances, with high ongoing exposure develops into desquamative interstitial pneumonia.[1,2] Such pulmonary disorder is commonly known as welder's lung or pulmonary siderosis. The transition from occupational respiratory bronchiolitis to desquamative interstitial pneumonia would occur following cumulative exposure to welding fume of the order of 100-200 mg/years/m^3. Bronchoalveolar lavage commonly reveals abundant hemosiderin-laden macrophages and lung biopsy hemosiderin pigment within alveolar macrophages and accumulation in an interstitial peri-broncho-vascular distribution.[3,4]

There are sporadic and incomplete descriptions of welders' siderosis associated with systemic iron overload. To the best of our knowledge, only few cases of welders' siderosis have been described in different reports suggesting that iron overload could be because of occupational exposure.[5,6] However, the evaluation of iron status in these patients was largely incomplete with no assessment of liver iron overload and distribution, liver function test and damage, and no extensive genetic testing of hemochromatosis-related genes. The present single-centre report of 20 welders with hepatic iron overload strongly supports the existence of a novel acquired, occupational form of hepatic iron overload associated with long exposure to welding fume.

2 | METHODS

2.1 | Patients

The 20 patients described in the paper represent a selected group of those who presented in the last ten years at the Centre for disorders of iron metabolism in Monza because of increased serum ferritin (s-FERR) of unknown origin, with or without high transferrin saturation (TSAT). According to the Centre[7] and the Regional diagnostic and therapeutic pathway (www.malattierare.marionegri.it), patients underwent specific diagnostic work-up as summarized below: (a) careful assessment of family history, patient's habits (smoke, alcohol intake), and working activity; (b) careful evaluation and exclusion of known secondary causes of iron overload (transfusions, iron loading anaemias, advanced cirrhosis, chronic liver diseases); (c) genetic testing for hemochromatosis-related genes (*HFE*, *HFE2*, *TFR2*, *HAMP*, *SLC40A1*); (d) assessment of hepatic iron overload by quantitative MRI and/or liver biopsy, if needed. Among a larger number of patients with a working history as wire and/or arc welders, we selected 20 patients who fulfil the following criteria: (a) presence of hepatic iron overload; (b) documented history of prolonged welder activity; (c) exclusion of Hemochromatosis; (d) exclusion of other inherited iron overload disorders including ferroportin disease and aceruloplasminemia; (e) exclusion of secondary causes of iron overload; (f) availability of a full set of indices of iron metabolism, liver function and metabolic tests, hepatic iron assessment, adequate amount of DNA samples.

Two control groups were constituted from our database. Controls for iron-related manifestations were 40 p. Cys282Tyr homozygous male patients matched for ferritin levels to each patient. Controls for serum hepcidin levels were 24 healthy male subjects matched for age selected from a larger group already presented elsewhere.[8]

2.2 | Methods

Age, smoking habits, alcohol intake and body mass index were collected at diagnosis. Baseline serum iron, transferrin and ferritin, liver function tests, blood counts, and metabolic tests were measured by standard methods. % of transferrin saturation (TSAT) was calculated as follows: [serum iron (μg/dL)/serum transferrin (mg/dL)*1.42]. Serum hepcidin was measured by ELISA kit according to the producer protocol (DRG Instruments Gmbh, Merburg, Germany) in 9 welders, 12 HFE-HH and 24 healthy controls.

Welders underwent evaluation by an occupational doctor to accurately collect information regarding duration of exposure on the job and use of personal protective devices (PPD), and a pulmonologist visit for clinical evaluation, pulmonary function tests (spirometry, diffusion lung CO (TLCO)), and high-resolution lung CT (HRCT). Ten patients underwent bronchoscopic examination using a flexible fiberoptic bronchoscope to collect bronchoalveolar lavage (BAL).

Assessment of liver iron overload was done prior to phlebotomy treatment. Nineteen welders underwent magnetic resonance assessment of liver iron (MRI^{-LIC}) according to Galimberti *et al*[9]; 11 of them also underwent liver biopsy; one patient was evaluated by liver biopsy alone. Thirty-one HFE-HH underwent liver biopsy, of whom 13 had also an MRI^{-LIC}; the other nine had MRI^{-LIC} alone. In 16 welders and 22 HFE-HH the T2* was measured also in the spleen and

the T2*Liver/T2*Spleen ratio was calculated. Specimens from liver biopsies were evaluated for morphology, staging and hemosiderin iron accumulation using standard and Perls' stains. Liver fibrosis was evaluated by Ishak's score,[10] and iron overload by semiquantitative score according to Deugnier *et al.*[11] Total iron score (TIS), hepatic iron scores (HIS), sinusoidal iron score (SIS) and portal iron score (PIS) were measured by an expert histologist. The SIS on TIS ratio was also calculated as reported.[12] Abdomen ultrasound was done to evaluate liver morphology, profile and structure, portal and spleen diameter.

All the patients were already tested for p. Cys282Tyr and p. His63Asp variants in *HFE* before counselling. Welders were then also tested for rare mutations in *HFE* and in genes causing non-HFE hemochromatosis (*HFE2*, *HAMP*, *TFR2* and *SLC40A1*) and Ferroportin disease (*SLC40A1*) according to recent reviews.[7,13] In brief, those with TSAT ≥ 45% underwent full sequencing of all genes, while those with TSAT < 45% were sequenced for *SLC40A1* alone.

Patients underwent phlebotomy treatment every 7 or 15 days as needed and total iron removed (IR) was calculated (400 mL of blood removed = 200 mg of iron). Although the ferritin level at which iron depletion was considered achieved was originally set around 50 ng/mL,[14] iron depletion in some welders was stopped before reaching this target if they showed persistent iron restricted erythropoiesis (TSAT < 16%) or mild anaemia after 15 days from the last phlebotomy (Table S1).

All medical procedures were done in accordance with international guidelines and diagnostic and therapeutic courses approved by the coordination Centre for Rare Disease of Regione Lombardia (http://malattierare.marionegri.it) for patients with undefined iron overload disorder and conform to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent for genetic testing and liver biopsy was obtained from each patient included in the study according to the ethical committee of the ASST-Monza, San Gerardo Hospital.

All calculations were performed using GraphPad Prism® 4.00 software. Continuous variables were expressed as medians with first and third quartiles. Quantitative data were analysed using the Student's t test or by analysis of variance (ANOVA), when appropriate. Qualitative data were analysed with the Chi-square or Fisher' test as appropriate. Correlations between quantitative variables were assessed using Pearson's coefficient test. Two-sided *P* values < 0.05 were considered statistically significant.

3 | RESULTS

Personal and occupational data of welders at diagnosis are reported in Table 1. All reported prolonged exposure to welding powders and 17 (85%) declared no or sporadic use of PPD. During the study, two

TABLE 1 Personal and occupational data of welders at diagnosis

Patient	Gender	Age (years)	BMI (kg/m^2)	Exposure (years)	PPD	Smoke habit (pack/year)
1	M	45	22.7	27	No/dis	0
2	M	38	25.0	24	No/dis	0
3	M	57	24.0	38	No/dis	45
4	M	45	21.4	15	No/dis	30
5	M	61	25.0	30	No/dis	0
6	M	51	26.1	25	No/dis	18
7	M	59	25.8	48	No/dis	40
8	M	63	24.8	39	No/dis	0
9	M	52	28.5	39	No/dis	0
10	M	57	21.9	40	No/dis	7.5
11	M	47	27.9	30	No/dis	0
12	M	52	20.7	28	Yes	36
13	M	46	25.7	31	No/dis	20
14	M	67	24.2	37	No/dis	0
15	M	70	26.6	40	No/dis	110
16	M	52	29.1	27	No/dis	80
17	M	57	25.3	20	No/dis	20
18	M	58	24.7	38	Yes	22.5
19	M	63	22.7	45	No/dis	112.5
20	M	47	24.9	24	Yes	56
Median		54.5	24.9	30	–	21.25
1st-3rd quartile		47-59.5	22.7-25.8	25-39	–	0-40

Note: BMI, Body Mass Index; PPD, Personal Protective Device; dis, discontinuous.

patients died, one for lung carcinoma whose association with welder fume is still debated,[2] and one for rapidly progressive amyotrophic lateral sclerosis.[15] One patient developed Parkinsonian symptoms and one cognitive impairment, findings that further support the higher risk of neurological impairment associated to welding fume exposure.[2]

3.1 | Iron status

All patients showed liver iron overload as assessed by MRI^{-LIC} or liver biopsy. There was no correlation between age, duration of fume exposure and smoking habits with liver iron overload. Eighteen welders (90%) had s-FERR above 1000 ng/mL and 12 (60%) had TSAT higher than 45%. TSAT significantly correlated with s-FERR (R^2 = 0.328, P <.01) and slightly with LIC (R^2 = 0.221, P <.05); s-FERR significantly correlated with MRI-LIC (R^2=0.60, P <.0001) and IR (R^2=0.49, P <.001). Interestingly, s-FERR correlated with TIS (R^2=0.36, P <.05) and mesenchymal iron (SIS + PIS) (R^2=0.59, P =.003), but not with HIS. Serum hepcidin significantly correlated with MRI-LIC (R^2=0.65, P =.008) and IR (R^2=0.63, P =.02) in welders (Figure 1). Of the 19 patients who underwent MRI, eight had MRI^{-LIC} below 100 μmol/g, five between 100 and 200 μmol/g, and six over 200 μmol/g; one patient who did not undergo MRI showed marked liver iron overload at biopsy. Overall, 8 (40%) had mild iron overload and 12 (60%) a moderate-severe one. The 12 patients that underwent liver biopsy showed mixed iron distribution involving hepatocytes, sinusoidal cell and portal spaces.

Table 2 reports main iron data of welders compared with the HFE-HH control group. Welders showed significantly lower TSAT than HFE-HH patients. MRI^{-LIC} was significantly lower in welders than in HFE-HH despite ferritin, TIS and total iron removed were comparable between the groups. However, while TIS did not significantly differ between the two groups, welders showed higher sinusoidal iron score (SIS) and SIS on TIS ratio than HFE-HH patients (Table 3). Accordingly, whereas MRI^{-LIC} correlated with TIS (R^2 = 0.57, P <.005), HIS (R^2 = 0.60, P <.002) and MIS (R^2=0.56, P <.005) in HFE-HH patients, it correlated with TIS (R^2=0.64, P =.002), MIS (R^2=0.77, P =.0003), but not with HIS in welders (Figure S1). The difference in liver iron distribution between welders and HFE-HH was also emphasized when comparing SIS according to HIS categories (Table 3), and by the T2*Liver/T2*Spleen ratio, which was significantly higher in welders than HFE-HH (Table 2). Finally, serum hepcidin levels in welders were significantly higher than in HFE-HH patients and healthy controls, as shown in Table 2.

3.2 | Iron depletion

Fifteen patients reached iron depletion after removal of a median of 7.8 g of iron (1st-3rd quartiles: 3.8-9.9), while the remaining five patients are still on treatment. More specifically, 3-5 g were removed from five patients, 5 to 10 g were removed from six, and more than 10 g from four patients. Iron removed correlated with s-FERR (R^2 = 0.46, P <.01) and with MRI^{-LIC} (R^2 = 0.49, P =.003). After iron depletion, haemoglobin, serum iron, TSAT, ferritin, AST, ALT, triglycerides and haemoglobin significantly decreased as reported in Table S1. No significant changes were observed for γGT, HDL cholesterol and glycaemia.

3.3 | Liver findings

Eight patients (40%) showed increased serum ALT. Thirteen patients (65%) showed liver steatosis at ultrasound, which was classified as mild in five patients, and moderate-severe in eight patients. Liver biopsy showed fatty infiltration in 8 of 12 patients (66.7%). It was mild (5%-15%) in four patients, moderate in two (30%) and severe in two (70% and 80% respectively). Concordance with ultrasound imaging was 75%, and non-concordance was limited to the cases with mild fatty infiltration. Patients with moderate-severe liver steatosis had higher serum ALT (54 U/L; 1st-3rd quartiles 39.5-72.5) compared with those with absent-mild fatty infiltration (31.5 U/L; 23.2-40.5) (P <.05). S-FERR was 2481 ng/mL (1st-3rd quartiles = 1358-2910 ng/mL) in those with moderate-severe steatosis compared to 1610 ng/mL (1203-2152 ng/mL), but the difference was not statistically significant. However, a mild correlation was present between s-FERR and grade of steatosis at ultrasound (R^2 = 0.23, P <.05), and between s-FERR and ALT (R^2 = 0.22, P <.05). Conversely, there were no correlations between fatty infiltration and alcohol intake, BMI and indices of the metabolic syndrome (HDL, glycemia, triglycerides). Liver fibrosis was present in six patients (50%): four of them had mild fibrosis (Ishak's staging 1-2), and 2 moderate-severe (staging 4). Liver staging correlated with TIS (R^2 = 0.41, P <.03), HIS (R^2 = 0.41, P <.03) and PIS (R^2 = 0.524, P <.01), but not with SIS.

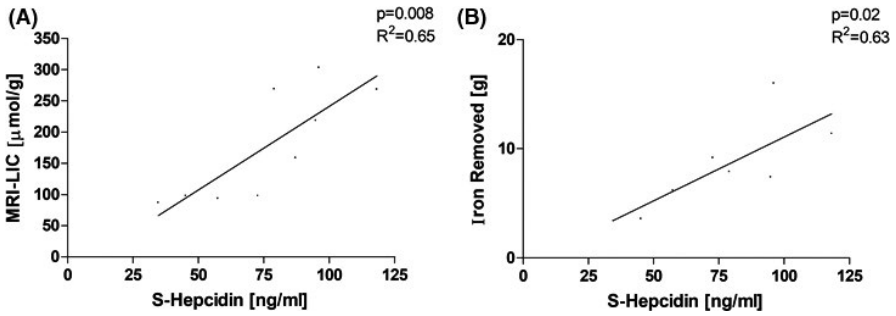

FIGURE 1 Correlation between serum hepcidin levels and iron parameters. (A) Serum hepcidin vs MRI. (B) Serum hepcidin vs Iron Removed (IR). Levels of significance: P =.008; P =.02

TABLE 2 Main indices in welders and HFE-HH

	Welders (N = 20)	HFE-HH patients (N = 40)	p
Age (years)	54.5 [47-59.5]	50 [40-59]	n.s.
Body Mass Index (kg/m^2)	24.9 [23.7-25.9]	25.4 [22.6-27]	n.s.
Alcohol intake (g/day)	11.5 [0-10]	10 [0-20]	n.s.
s-Ferritin (μg/L)	1770 [1251-2634]	1617 [1131-2520]	n.s.
Transferrin saturation (%)	53 [39-63]	84.5 [75.5-90.2]	< 0.0001
s-Hepcidin (ng/mL)†	72.7 [47.4-95.4]	12.75 [10.3-19.3]	= 0.003
Liver Iron Concentration* (μg/g)	108.2 [88.3-191.5]	294.3 [180.7-370.8]	< 0.001
Iron Removed (g)	7.8 [3.9-10.7]	8.3 [5.3-11]	n.s.
T2* Liver (msec)	4.1 [2.7-5.1]	1.7 [1.25-3.15]	= 0.0011
T2* Spleen (msec)	5.75 [3.5-7.9]	23 [20.2-29.5]	< 0.0000
T2* Liver/Spleen ratio§	0.67 [0.57-0.82]	0.1 [0.05-0.11]	< 0.0000
Semiquantitative Liver Iron Score°			
▪ *Total Iron Score (TIS)*	31 [28.8-35]	36 [25-40.5]	n.s.
▪ *Hepatic Iron Score (HIS)*	21 [18-21]	21 [19.5-24]	n.s.
▪ *Sinusoidal Iron Score (SIS)*	6.5 [6.0-8.2]	6 [3-6.5]	= 0.013
▪ *Portal Iron Score (PIS)*	4 [2.5-6.5]	7 [2.5-11]	= 0.05
▪ *Ratio SIS/TIS*	0.23 [0.19-0.27]	0.16 [0.13-0.17]	< 0.001

Note: Data are expressed as median [1st- 3rd quartiles].

† done in 9 welders and 7 HFE-HH.

*done in 18 welders and 22 HFE-HH.

§done in 16 welders and 22 HFE-HH.

°done in 12 welders and 31 HFE-HH.

TABLE 3 Sinusoidal Iron Score (SIS) in welders and HFE-HH patients according to Hepatic Iron Score (HIS) categories

Hepatic Iron Score	Sinusoidal Iron Score		
	HFE-HH	Welders	*P*
HIS < 21	3 (3-3)	7.5 (6-9.25)	<0.01
HIS ≥ 21	6 (5.5-7.5)	6.5 (6-7.259)	n.s.
P	0.001	n.s.	

Note: Data are expressed as median [1st-3rd].

3.4 | Pulmonary findings

Three patients showed reduced forced expiratory volume (FEV1 < 65%), three showed TLCO < 65% and three between 70% - 80%. Spirometry pulmonary function tests inversely correlated with years of fume exposure (FEV1: $R^2 = 0.303$, $P = .012$; FVC: $R^2 = 0.27$, $P < .02$), and packs/year smoking (FEV1: $R^2 = 0.28$, $P < .02$; FEV1 (%):$R^2 = 0.43$, $P < .002$; FEV1/FVC: $R^2 = 0.34$, $P < .01$). Patients with prolonged fume exposure had significantly lower FEV1 and FVC than patients with shorter exposures, and smokers had lower FEV1 and TLCO as compared to non-smokers (Table S2). The great majority showed lung HRCT alterations (Table S3). All of the 10 patients who underwent to bronchoscopy showed 80 to 100% iron-laden macrophages at BAL.

3.5 | Genetic testing

Three patients were heterozygous for p. His63Asp variant in *HFE* and one heterozygous for p. Cys282Tyr. These frequencies were slightly lower and higher than in healthy Italian population (p. His63Asp heterozygotes = 15% vs 21%; p. Cys282Tyr heterozygotes = 5% vs 2%, respectively), but not significantly different.[16] No causal mutations were identified in the other hemochromatosis-related genes (*TFR2*, *SLC40A1*, *HAMP*, *HFE2*).

4 | DISCUSSION

Our study provides novel findings which support the notion that welders with prolonged fume exposure can develop systemic iron overload and liver damage. Firstly, we collected a number of welders who unequivocally showed hepatic iron overload that varied from mild-moderate to moderate-severe; we also showed that they shared a prolonged exposure to welder fume, and that the majority declared no or sporadic use of PPD. Secondly, none of the patients studied had any of the known genetic or acquired cause of iron overload. Thirdly, patients showed mixed liver iron distribution with early and prevalent sinusoidal iron accumulation, and 40% had normal TSAT at diagnosis. Fourthly, 50% of patients who underwent to liver biopsy showed fibrosis that was mild in 33%, and moderate-severe in 17%,

and correlated with iron overload. Fifthly, a large number of patients presented fatty infiltration that did not correlate with metabolic indices. This allows the following conclusions to be drawn: (a) prolonged exposure to welder fume should be considered an additional cause of liver iron overload and information regarding employment in welding activities should be part of the evaluation of patients presenting with hyperferritinemia; (b) liver iron accumulation does not follow the typical hemochromatosis pattern in these patients; (c) liver iron accumulation can exceed the threshold of iron toxicity and if untreated can lead to liver damage, thus adding another complication to the other chronic adverse events that involve respiratory, neurological, skin and reproductive systems[1,2]; (d) the high prevalence of fatty liver infiltration and the lack of correlations with metabolic indices suggests that iron itself or other components of welder fume could be involved, and that it can be implicated as a cofactor of liver damage together with iron.

This study does not clarify how a focal iron overload confined to alveolar macrophages and lung interstitium, as it is currently considered welder siderosis, can evolve into systemic iron overload. Morphological characterizations of welding fume have shown that many of the individual particles are in the ultrafine size range (0.01-0.10 μm). Iron handling in the lung is mainly focused on sequestering and detoxifying breathing-derived iron through pathways that include both metal importation and exportation.[17-19] Divalent metal transporter 1, ZIP14, ferritin and ferroportin coordinate with the aim to transport iron of the lung via the mucociliary pathway or blood and lymphatic pathways to the reticuloendothelial system. The net rate of alveolar deposition of particles per year in full-time welders was estimated at 70 mg of iron per year, and after 10 years of welding the average burden of ferrous metal particles in the lungs was 1 g[2]. Because our patients showed liver iron overload that largely overcomes this number, we can speculate from here that ultrafine iron particles can access the body systemically through different ways. Al-Shamma *et al*[20] observed that the main elimination route for the different components of welding fumes was via the gastrointestinal tract, and that some systemic distribution of soluble constituents (such as iron, chromium, cobalt and nickel) occurred via the blood, thus posing the question whether long-term accumulation of different metals arising from the inhalation of welding fumes might lead to significant toxicity of various vital organ systems, such as the brain/central nervous system. Moreover, welding fume microparticles including iron can spread and stick to the mouth or nasal mucosa and be slowly ingested with salivary secretion. We can hypothesize that some iron particles are absorbed although how this occurs remains to be elucidated. Recently, studies aimed at identifying new iron formulations to correct iron deficiency showed than nanoparticulate iron can be absorbed by intestinal mucosa via endocytosis.[21] Following lysosomal dissolution, the iron derived from nano-iron joins the common enterocyte iron pool and is exported to the systemic circulation via ferroportin.[22] We can speculate that this can also occur for the ultrafine welding fume particles, and if this is the prevalent way of iron accumulation in welders, we should expect high TSAT and prevalent hepatocellular iron overload as in hemochromatosis. However, this only partially fits with our findings in these patients. In fact: (i) 40% of the patients had normal TSAT despite increased s-FERR and liver iron accumulation; (ii) patients with moderate iron overload (MRI-^{LIC} < 100 μmol/g) showed lower TSAT [median (1st-3rd quartile) = 38% (34.2%-44.2%)] compared to 54% (50.7%-67.7%) in those with MRI-^{LIC} > 100 μmol/g (P <.005), suggesting that TSAT increased with the increasing iron accumulation; (iii) SIS and SIS/TIS ratio were higher than in HFE-HH; (iv) while SIS increased progressively with the increasing HIS in HFE-HH, as expected, welders showed high SIS even in the lower HIS category (Table 3); (v) There was a significantly higher spleen iron accumulation in welders than in HFE-HH as measured by MRI. These findings indicate that the mechanism of liver iron accumulation is quite different to that of HFE-HH suggesting that reticuloendothelial cells is the initial site of deposition and that hepatocyte iron loading progresses as reticuloendothelial system is saturated, similar to what occurs in transfusion-dependent iron overload.[23-25] The greater involvement of reticuloendothelial cells and spleen might also explain the lower LIC in welders compared to HFE-HH despite comparable levels of serum ferritin, TIS and IR. The higher concentration of serum hepcidin in welders than in HFE-HH and healthy controls, and the correlations between serum hepcidin and indices of liver and body iron overload, further highlight the difference between welders and HFE-HH, indicating that the iron-dependent hepcidin regulation is overall preserved in welders, and that iron accumulation should be mainly classified among the acquired forms of iron overload. Last, the correlation of TIS and PIS with liver staging indicates that iron toxicity and liver damage develop as liver iron accumulation increases progressively involving portal tracts. Although these data could reflect the overall severity of iron overload and the duration of the disease, they might suggest a role for macrophagic iron in hepatic fibrogenesis in agreement with that observed in hemochromatosis[11] and other histopathological and experimental studies.[26] The improvement of serum transaminases and triglycerides after iron depletion (Table S1) supports a role for iron in hepatic damage and lipid derangement as observed in other iron overload disorders.[27]

In Italy the number of workers employed in welding activities (manufacturing metal products, machines and mechanical appliances) is still very high, and an estimated 800,000 workers are employed full time as welders worldwide, with more than one million performing welding intermittently as part of their work duties.[2] The risks of this activity, which employs different technologies, depend on many factors: the technology used, the materials used, the workplace, the type and intensity of the exposure. Most of the materials in the welding fume come from the consumable electrode, which is partially volatilized in the welding process; a small fraction of the fume is derived from spattered particles and the molten welding pool.[2] Most welding materials are alloy mixtures of metals characterized by different steels that may contain iron, manganese, silica, chromium, nickel and others, and the rate at which fumes are generated by a welding arc is dependent on the process, current level and the compositions of the wire/flux used as the consumable electrode.[2] Thus, welders are not a homogeneous group regarding their risk to pathologies, and

this might also explain the variability in the development of systemic iron overload in welders. Casjens *et al.*[28] found increased s-FERR levels (cut-off: >300 ng/mL) in 32/192 (16.7%) welders in Germany. Although an exhaustive characterization of iron overload was lacking, they found slightly significant higher levels of s-FERR in those with substantial exposure to respirable iron in welding fume compared to all other welders (median: 241 vs 121 ng/mL) and a higher prevalence of s-FERR > 300 ng/mL [8/21 (38.1%) vs 24/171 (14.7%)] respectively. Similar findings were reported in 97 welders in China,[29] showing 21% serum iron and 18% s-FERR increase compared to matched controls, an increase that was associated with the welder's professional years and suggested a systemic overload of iron. However, the amount of iron overload, as based on the level of s-FERR, was much lower than that reported in our series. One possible explanation is the younger age of welders (10-15 years younger) and their lower average employment history (10-15 years less) compared to our patients. However, as only a minor proportion of welders develop significant systemic iron overload, it is likely that factors other than respirable iron in fume and cigarettes are involved, suggesting a multifactorial susceptibility, which may also include genetic components, favouring the absorption of iron nanoparticles and iron accumulation.

In conclusion, the present study shows that a subgroup of welders with prolonged exposure to welding fume, and particularly those with less use of protective devices can develop severe systemic iron overload requiring iron depletion to prevent or rescue from iron-related damage. We recommend that measurement of serum iron indices should be part of the routine test in welders to identify subjects at risk and to provide adequate measures for preventing and controlling occupational exposure to iron. We also recommend that prolonged exposure to welding fume is included in the list of rare and acquired causes of hyperferritinemia and liver iron overload. Finally, considering the multiorgan involvement (lung, liver and neurological system) that we observed in our patients, we recommend a multidisciplinary evaluation of the patients that, in our experience, were only partially aware of the full risks related to their professional activity.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of ASST Monza under the General Data Protection Regulation (GDPR) 679/2016 and Legislative Decree 101/2018 (protocol code GEN-CI-001A dated 10/12/2018 for genetical analysis; PN-FI-002 rev1 del 29/04/2015 for pneumological procedures; ASST-MA-002 del 15/05/2017 for liver biopsy and MRI).

5 | INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

AUTHORS CONTRIBUTIONS

Study concept and design: RM, APi; acquisition of data: VP, MB, FDG, PF, MR; analysis and interpretation of data: RM, APe, APi; drafting of the manuscript: RM, SP, APi; critical revision of the manuscript for important intellectual content: RM, MR, APe, APi; statistical analysis: SP; administrative, technical or material support: SP; writing—review and editing: RM, SP, VP, MB, FDG, PF, MR, APe, APi; study supervision: APi. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Not applicable.

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REFERENCES

1. Cosgrove MP. Pulmonary fibrosis and exposure to steel welding fume. *Occup Med (Lond)*. 2015;65(9):706-712.
2. Antonini JM. Health effects of welding. *Crit Rev Toxicol*. 2003;33(1):61-103.
3. Modrykamien A, Christie H, Farver C, Ashton RW. A 38-year-old welder with dyspnea and iron overload. *Chest*. 2009;136(1):310-313.
4. McCormick LM, Goddard M, Mahadeva R. Pulmonary fibrosis secondary to siderosis causing symptomatic respiratory disease: a case report. *J Med Case Rep*. 2008;2:257.
5. Patel RR, Yi ES, Ryu JH. Systemic iron overload associated with Welder's siderosis. *Am J Med Sci*. 2009;337(1):57-59.
6. Doherty MJ, Healy M, Richardson SG, Fisher NC. Total body iron overload in welder's siderosis. *Occup Environ Med*. 2004;61(1):82-85.
7. Piperno A, Pelucchi S, Mariani R. Inherited iron overload disorders. *Transl Gastroenterol Hepatol*. 2020;5:25.
8. Piperno A, Galimberti S, Mariani R, et al. Modulation of hepcidin production during hypoxia-induced erythropoiesis in humans in vivo: data from the HIGHCARE project. *Blood*. 2011;117(10):2953-2959.
9. Galimberti S, Trombini P, Bernasconi DP, et al. Simultaneous liver iron and fat measures by magnetic resonance imaging in patients with hyperferritinemia. *Scand J Gastroenterol*. 2015;50(4):429-438.
10. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol*. 1995;22(6):696-699.
11. Deugnier YM, Loréal O, Turlin B, et al. Liver pathology in genetic hemochromatosis: a review of 135 homozygous cases and their bioclinical correlations. *Gastroenterology*. 1992;102(6):2050-2059.
12. Riva A, Trombini P, Mariani R, et al. Revaluation of clinical and histological criteria for diagnosis of dysmetabolic iron overload syndrome. *World J Gastroenterol*. 2008;14(30):4745-4752.
13. Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loréal O. Haemochromatosis. *Nat Rev Dis Primers*. 2018;4:18016.

14. Adams P, Altes A, Brissot P, et al. Therapeutic recommendations in HFE hemochromatosis for p.Cys282Tyr (C282Y/C282Y) homozygous genotype. *Hepatol Int*. 2018;12(2):83-86.
15. Strickland D, Smith SA, Dolliff G, Goldman L, Roelofs RI. Amyotrophic lateral sclerosis and occupational history. A pilot case-control study. *Arch Neurol*. 1996;53(8):730-733.
16. Piperno A, Sampietro M, Pietrangelo A, et al. Heterogeneity of hemochromatosis in Italy. *Gastroenterology*. 1998;114(5):996-1002.
17. Giorgi G, D'Anna MC, Roque ME. Iron homeostasis and its disruption in mouse lung in iron deficiency and overload. *Exp Physiol*. 2015;100(10):1199-1216.
18. Ghio AJ. Disruption of iron homeostasis and lung disease. *Biochim Biophys Acta*. 2009;1790(7):731-739.
19. Nguyen NB, Callaghan KD, Ghio AJ, Haile DJ, Yang F. Hepcidin expression and iron transport in alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol*. 2006;291(3):L417-425.
20. Al-Shamma KJ, Hewitt PJ, Hicks R. The elimination and distribution of components of welding fumes from lung deposits in the guinea pig. *Ann Occup Hyg*. 1979;22(1):33-41.
21. Pereira DIA, Bruggraber SFA, Faria N, et al. Nanoparticulate iron(III) oxo-hydroxide delivers safe iron that is well absorbed and utilised in humans. *Nanomedicine*. 2014;10(8):1877-1886.
22. Aslam MF, Frazer DM, Faria N, et al. Ferroportin mediates the intestinal absorption of iron from a nanoparticulate ferritin core mimetic in mice. *FASEB J*. 2014;28(8):3671-3678.
23. Porter JB, Walter PB, Neumayr LD, et al. Mechanisms of plasma non-transferrin bound iron generation: insights from comparing transfused diamond blackfan anaemia with sickle cell and thalassaemia patients. *Br J Haematol*. 2014;167(5):692-696.
24. Ghugre NR, Gonzalez-Gomez I, Butensky E, et al. Patterns of hepatic iron distribution in patients with chronically transfused thalassemia and sickle cell disease. *Am J Hematol*. 2009;84(8):480-483.
25. Mariani R, Trombini P, Pozzi M, Piperno A. Iron metabolism in thalassemia and sickle cell disease. *Mediterr J Hematol Infect Dis*. 2009;1(1):e2009006.
26. Corengia C, Galimberti S, Bovo G, et al. Iron accumulation in chronic hepatitis C: relation of hepatic iron distribution, HFE genotype, and disease course. *Am J Clin Pathol*. 2005;124(6):846-853.
27. Piperno A, Vergani A, Salvioni A, et al. Effects of venesections and restricted diet in patients with the insulin-resistance hepatic iron overload syndrome. *Liver Int*. 2004;24(5):471-476.
28. Casjens S, Henry J, Rihs H-P, et al. Influence of welding fume on systemic iron status. *Ann Occup Hyg*. 2014;58(9):1143-1154.
29. Lu L, Zhang LL, Li GJ, Guo W, Liang W, Zheng W. Alteration of serum concentrations of manganese, iron, ferritin, and transferrin receptor following exposure to welding fumes among career welders. *Neurotoxicology*. 2005;26(2):257-265.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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