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ScienceDirect
Forensic Science International 173 (2007) 73–77
Forensic Science International
www.elsevier.com/locate/forsciint

Case report

# An immunohistochemical study in a fatal case of acute interstitial pneumonitis (Hamman–Rich syndrome) in a 15-year-old boy presenting as sudden death

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Received 27 March 2006; received in revised form 20 November 2006; accepted 5 December 2006
Available online 8 January 2007

**Abstract**

Acute interstitial pneumonitis (AIP), also known as Hamman–Rich syndrome, is a distinct type of idiopathic interstitial pneumonia affecting patients of both genders without pre-existing lung diseases. We describe the case of a fulminant form of AIP and discuss the pathophysiological mechanisms of AIP with reference to the histological pattern. A 15-year-previously-healthy male boy presented to the Hospital with a 6-day history of malaise, fever and cough. The clinical prodromes were followed by the acute onset of increasing shortness of breath rapidly progressing in acute respiratory failure. Chest X-ray demonstrated bilateral diffuse airspace opacification; the high resolution CT confirmed the presence of bilateral, symmetric diffuse ground-glass attenuation. The patient was admitted to the intensive care unit, but died after few hours.

An autopsy was performed within 24 h. The histological examination of lung specimens showed a pattern of diffuse alveolar damage. immunohistochemical, microbiological and toxicological tests were also carried out. The clinical presentation, the histological findings and the exclusion of infective, traumatic, toxic and metabolic causes of acute respiratory distress syndrome (ARDS) allowed us to conclude that the boy was affected by AIP.

In conclusion, AIP is a diagnosis of exclusion. It has a mortality rate ranging about 50%, despite mechanical ventilation. In fatal cases of AIP diagnosis can be based on clinical presentation, radiological, histological and microbiological findings and can be further confirmed by immunohistochemical analysis.


*Keywords:* Acute interstitial pneumonitis; Hamman–Rich syndrome; Adult respiratory distress syndrome; Diffuse alveolar damage; Immunohistochemistry; Anti-surfactant apoprotein (PE-10); Cytokines

## 1. Introduction

Acute interstitial pneumonitis (AIP) is a fulminant disease culminating in acute respiratory failure and often death [1]. In 1935, Hamman and Rich were the first who described few fatal cases of fulminant pulmonary failure, histologically characterized by ‘‘a peculiar, progressive, diffuse fibrosis of the pulmonary alveolar walls’’ [2,3]. The linking features of these cases were, more than the histological pattern, the previously healthy condition of all patients and the history of a viral-like prodromes which rapidly progressed in fatal acute respiratory failure, within 6 months after onset of symptoms. They termed this condition ‘‘acute diffuse interstitial fibrosis of the lungs.’’

In 1986, Katzenstein et al. [4,5] coined the term ‘‘Acute Interstitial Pneumonitis’’ to describe the idiopathic interstitial lung disease they have observed in eight patients causing abrupt onset of respiratory failure, and to distinguish this clinico-pathological entity from chronic interstitial pneumonias.

In 1990, Olson et al. [6], reviewing and comparing the autopsy material of Hamman–Rich and Katzenstein, concluded that AIP represented the same pathological process described by Hamman and Rich, and distinguished AIP from many other clinicopathological entities inappropriately termed ‘‘Hamman–Rich syndrome,’’ like secondary Acute Interstitial Pneumonias and exacerbations of cryptogenic fibrosing alveolitis [7,8]. AIP is a rapidly progressive respiratory failure occurring in patients without pre-existing lung disease; typically it shows a flu-like

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doi:10.1016/j.forsciint.2006.12.002

onset. The radiological and histological patterns are those of acute respiratory distress syndrome (ARDS); however, AIP differs from ARDS for the absence of known inciting events, and the lack of multiorgan involvement. While mortality in all patients with ARDS has decreased to 31%, there have been no analogous reports of an improvement in mortality rate in patients with AIP, wherein every reported series the hospital mortality has been >50% [9].

We report the case of a previously healthy 15-year-old boy, who died in the extremely rapid course of few hours from a rapidly progressive respiratory failure of unknown cause. The clinical course, the CT findings, and the post-mortem examination with histological, immunohistochemical and microbiological exams, lead us to conclude he was affected by AIP. The features of the disease are discussed with reference to the histological and immunohistochemical evaluation.

## 2. Case report

### 2.1. Clinical history

A previously healthy 15-year-old non-smoking male was admitted to the Hospital with a 7-day history of flu-like symptoms (malaise, inappetence, cough, fever with a temperature of 39 °C) treated with antipyretic drugs.

On admission the patient presented marked asthenia, hypotension and dehydration, so he was administered supportive and antibiotic therapy. Two hours after admission his condition got worst with the acute onset of dyspnoea and rapidly progressive respiratory failure, requiring intubation and mechanical ventilation. On physical examination he presented tachycardia, hypotension, haemoptysis, cyanosis, diffusely reduced vesicular murmur, crackles, wheezing. The haemogasanalysis showed an important hypoxemia (pH 7.22; $pO_2$ 21 mmHg; $pCO_2$ 32 mmHg), neutrophilic leukocytosis (WBC 21.100/μl, neutrophils 89.3%). Despite intravenous antibiotics no significant improvement was observed. Chest X-ray demonstrated bilateral diffuse airspace opacification; the high resolution CT confirmed the presence of bilateral, symmetric diffuse ground-glass attenuation. The patient underwent in a comatose state (pH 6.868, $pCO_2$ 50.1 mmHg, $pO_2$ 41 mmHg; 34.900/μl, neutrophils 86.7%). After an episode of ventricular fibrillation, despite the resuscitative maneuvers, the boy died from cardiac arrest 6 h after the admission.

### 2.2. Autoptic findings

The autopsy was performed 24 h after death. The body was that of a well-developed 15-year-old boy. Pleural cavities contained 300 $cm^3$ of reddish fluid mixed with fibrin. Macroscopically lungs appeared hypoexpanded and heavy (1050 g the left and 1020 g the right, respectively) with diffuse, firm and red boggy parenchyma. The examination of other organs was unremarkable except for cerebral oedema, cardiac petechiae and intense polyvisceral stasis.

### 2.3. Histological studies

A routine microscopic histopathological study was performed using haematoxylin–eosin, Wilder (silver impregnation for reticular fibers), PAS, Grocott, Gram, Ziehel–Neelsen (for bacteria and fungi) stainings. An immunohistochemical investigation of lung samples was performed utilizing antibodies anti-surfactant apoprotein (PE-10), MCP-1, IL-6, IL-8, IL-10, TNF-α, RSV, HSV1, HSV2, VZV, CMV, Adenovirus, *Aspergillus* spp., *Pneumocystis carinii*, *Toxoplasma gondii*. We used 4 μm thick paraffin embedded sections, mounted on slides covered with 3-amminopropyltriethoxysilane (Fluka, Buchs, Switzerland). A pre-treatment was necessary, for some antibody, to facilitate antigen retrieval and to increase membrane permeability to antibodies The primary antibody was applied and incubated, at 20 °C (see Table 1).

The detection system utilized was the LSAB+ kit (Dako, Netherlands), a refined avidin–biotin technique in which a biotinylated secondary antibody reacts with several peroxidase-conjugated streptavidin molecules. The positive reaction was visualized by 3,3-diaminobenzidine (DAB) peroxidation,

Table 1
Panel of investigated antibodies

| Antibody against | Pre-treatment | Incubation time of primary antibody, temperature | Concentration of primary antibody |
|---|---|---|---|
| IL-6 (Santa Crouz, USA) | 5 min proteolytic enzyme (Dako, Netherlands), 20 °C | 120 min, 20 °C | 1:2000 |
| IL-8 (Abcam, UK) | 5 min proteolytic enzyme (Dako, Netherlands), 20 °C | 120 min, 20 °C | 1:500 |
| IL-10 (Peprotec, UK) | 5 min proteolytic enzyme (Dako, Netherlands), 20 °C | 120 min, 20 °C | 1:4000 |
| TNF-α (Santa Crouz, USA) | Boiling in 0.1 M citric acid buffer | 120 min, 20 °C | 1:600 |
| MCP-1 (Santa Crouz, USA) | Boiling in 0.25 mM EDTA buffer | 120 min, 20 °C | 1:500 |
| S-AP-PE-10 (Dako, Denmark) | No pre-treatment | 120 min, 20 °C | 1:50 |
| RSV (Abcam, UK) | Boiling in 0.25 mM EDTA buffer | Overnight, 20 °C | 1:40 |
| HSV 1–2 (Dako, Netherlands) | 5 min proteolytic enzyme (Dako, Netherlands), 20 °C | 120 min, 20 °C | 1:200 |
| Adenovirus (Abcam, UK) | Boiling in 0.1 M citric acid buffer | 120 min, 20 °C | 1:100 |
| Citomegalovirus (Dako, Netherlands) | Boiling in 0.25 mM EDTA buffer | 120 min, 20 °C | 1:20 |
| Varicella Zoster Virus (Abcam, UK) | Boiling in 0.1 M citric acid buffer | Overnight, 20 °C | 1:50 |
| *Aspergillus* spp. (Dako, Netherlands) | Boiling in 0.25 mM EDTA buffer | Overnight, 20 °C | 1:50 |
| *P. carinii* (Dako, Netherlands) | No pre-treatment | Overnight, 20 °C | 1:20 |
| *T. gondii* (Abcam, UK) | No pre-treatment | 120 min, 20 °C | 1:200 |