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Low molecular weight heparin as cause of liver injury: case report and literature review

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Abstract. – Low molecular weight heparins (LMWH) are a class of drugs including various molecules that inhibit predominantly the factor V of coagulation and are used in a wide range of clinical settings for the management of venous thromboembolism and acute coronary syndrome. Despite LMWH are considered safe and associated with a lower incidence of side effects compared to unfractioned heparin, it is worth considering that the use of LWMH can be associated with complications. Some of these, such as bleeding and thrombocytopenia, are well-known, whereas other ones are often underestimated leading to a diagnostic delay. In this case report, we describe a case of a 73-years-old man who recently started nadroparin for deep vein thrombosis presenting with acute hepatitis. The diagnostic workup of drug-induced liver injury (DILI) requires the exclusion of other causative agents and temporal association between the initiation of the culprit drug and hyper aminotransferasemia. This clinical case analyzes how to deal with a suspicion of DILI and consider LWMH as a potential cause of DILI, which requires a modification of the anticoagulant treatment.

Key Words:
Drug induced liver injury, Low molecular weight heparin, Nadroparin, Venous thromboembolism, Liver histopathology.

Case Report

A 73-year-old man with a thrombosis of the popliteal vein started anticoagulant therapy with enoxaparin 0.4 ml/4000 IU bid (1 mg/kg twice daily) for 6 days and then switched to nadroparin 0.6 ml bid. His chronic therapy also included clopidogrel, simvastatin (started in 2001 after revascularization of the left iliac artery), and esomeprazole. He also took ketorolac (3 doses) for abdominal pain. He reported consumption of 2 alcohol units a day and denied any binge drinking in his recent past. The liver tests before starting anticoagulant treatment were completely normal [aspartate aminotransferase (AST) 21/40 IU/L, alanine aminotransferase (ALT) 24/40 IU/L, total bilirubin 0.89 mg/dl]. The patient did not have history of liver disease or use of herbal medicines. A few days after the start of low molecular weight heparin (LWMH), he complained of progressive fatigue without any improvement in abdominal pain: two weeks later, the blood tests showed an increase in the serum aminotransferases and cholestasis [AST 147/34 IU/L, ALT 338/45 IU/L, gamma glutamyl transferase (GGT) 91/73 IU/L, alkaline phosphatase (ALP) 133/129 IU/L], with normal bilirubin levels (Figure 1). The aminotransferases serum levels further deteriorated 3 days later (ALT 2175/45, AST 796/34), suggesting a pattern of hepatocellular injury [ratio ALT/ALP (R) > 5] accompanied by a slight alteration of the normalized internationalized ratio (1.11) and serum albumin (3.3 mg/dl) without jaundice. The blood tests [hepatitis A (HAV), B (HBV), C (HCV), and E (HEV), cytomegalovirus (CMV), total IgM and IgG, anti-nucleus antibodies (ANA), anti-smooth muscle antibodies (ASMA), anti-liver-kidney antibodies (LKM)] excluded viral and autoimmune etiology of liver damage. The abdominal ultrasound examination showed mild steatosis. A computed tomography (CT) scan showed hepatomegaly with hypertro-

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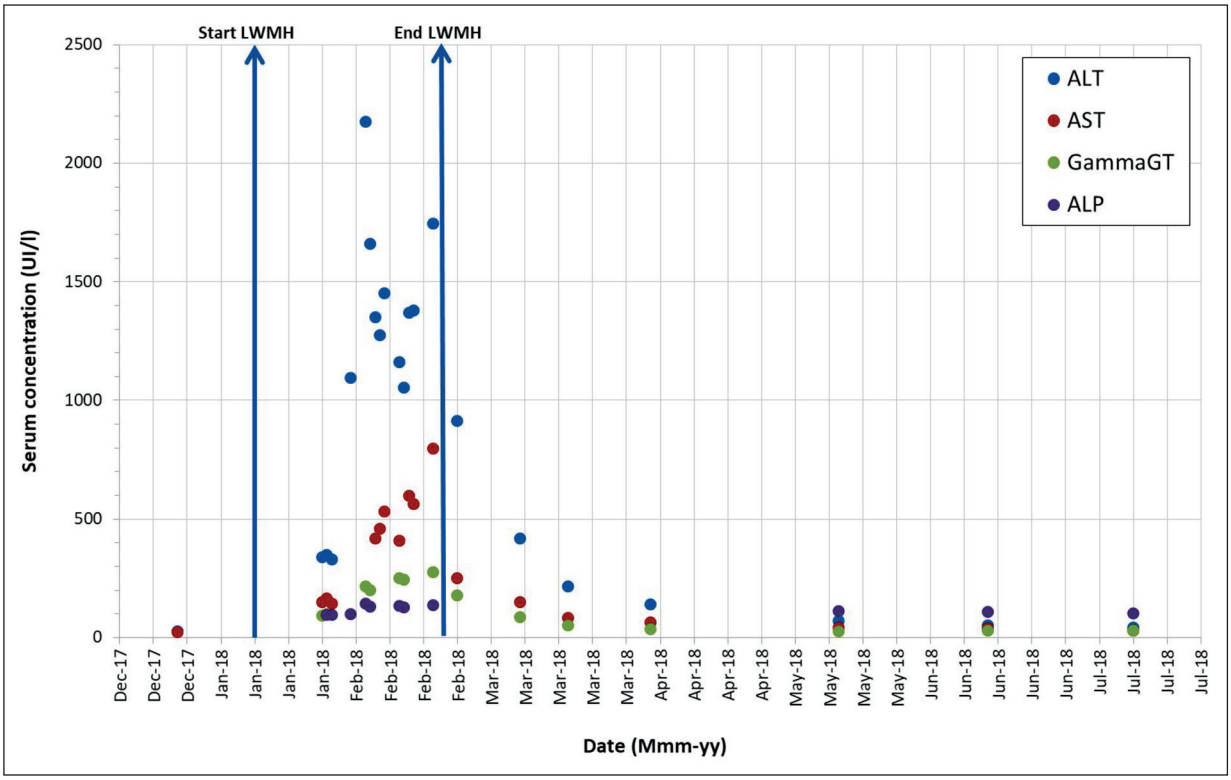

Figure 1. Trend of liver function test during patient's follow-up.

phy of the left lobe without any significant focal lesion or alteration of the vascular or biliary tree. A liver biopsy was then performed, showing minimal hepatocytes hemosiderosis and biliary metaplasia, without any significant enlargement of the portal tracts. Several hepatocytes showed a ground glass appearance of the cytoplasm (Figure 2). This was due to pale, homogeneous, weakly eosinophilic inclusions that filled a portion of or the entire cytoplasm of hepatocytes. HBV surface antigen (HBsAg) and periodic acid-Schiff (PAS) staining were negative, while immunohistochemistry revealed selective and exclusive positivity for fibrinogen. The Roussel Uclaf Causality Assessment Method (RUCAM) score was 9; therefore, due to the suspicion of DILI, the statin treatment was discontinued without significant improvement in laboratory tests. Nadroparin was then discontinued and anticoagulant therapy with a vitamin K inhibitor was initiated. Five months after the suspension, the serum aminotransferases gradually decreased until complete normalization.

Comments

Drug induced liver injury (DILI) is caused by exposure to a drug or a non-infectious toxic agent with a varying degree of organ dysfunction. It can be classified in two distinct types: intrinsic and idiosyncratic. The first is characterized by a predictable dose-dependent acute liver damage with a short time of onset (hours), which occurs when a known responsible agent (e.g., acetaminophen) is administered; the sec-

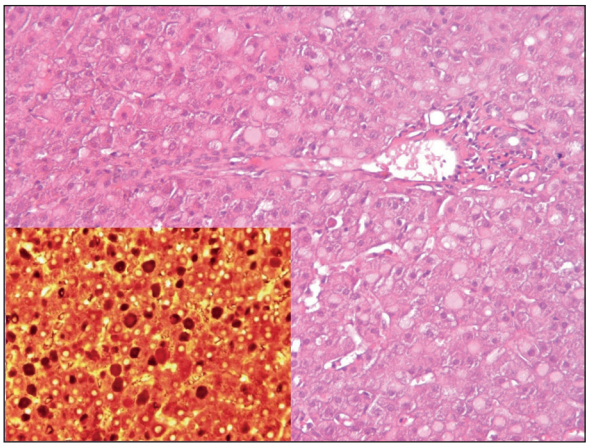

Figure 2. Liver biopsy showing numerous ground glass hepatocytes (*arrows*) with cytoplasmic inclusions due to fibrinogen storage. Hematoxylin and eosin (H&E) and immunohistochemical stain for fibrinogen (*insert*). 20× original magnification.

ond, instead, is rare (the estimated overall annual incidence is 19.1 cases per 100,000 persons)[1,2], unpredictable, and dose independent. Idiosyncratic DILI has a variable time of onset and extent of damage, and is potentially related to the assumption of any type of drug, reflecting an inter-individual susceptibility. It is divided into two forms: a) hypersensitivity-based, which is mediated by an aberrant immune response and is usually accompanied by systemic clinical features such as fever, rash, eosinophilia, and arthralgia; b) toxic metabolite-dependent, which recognizes the cause of liver injury in a toxic reactive metabolite of the drug. However, despite the diagnostic efforts, in most cases the exact cause of DILI remains undefined. DILI can resolve without consequences or be life threatening: the hepatocellular pattern is associated with a worse outcome (7-13%) while the mixed pattern has the lowest incidence of adverse outcomes (2%); hepatic lesions induced by isoniazid and halothane are burdened by 40% rate of death or transplantation. A "red flag" is the development of jaundice [total serum bilirubin greater than 2 × normal upper limit (ULN)] in a hepatocellular lesion pattern without cholestasis; this is otherwise known as Hy's law, and is associated with a mortality of 10-50%[3]. Approximately 10% of these patients can progress to acute liver failure with a mortality rate up to 80%. The first step in the diagnostic workup of DILI is to determine the pattern of damage from laboratory tests, calculating the "R" value, which is the ratio between the serum activity of ALT and ALP, expressed as a multiple of the ULN. When there is an increase in ALT above 3 × ULN with a normal ALP or when the ratio is ≥ 5, DILI is designated as "hepatocellular". An increase of more than 2 × ULN in ALP with a normal ALT or a R ≤ 2 defines a "cholestatic" DILI, while a "mixed" pattern is characterized by an increase of more than 2 × ULN in ALT, an increase in ALP and a R between 2 and 5[4]. Heparin administration has been reported to cause mainly hepatocellular damage pattern, although cholestatic DILI has also been reported[5,6]. In our case, the R-value was > 5, which was consistent with the typical presentation of heparin-induced liver injury. The second step is the exclusion of viral, metabolic, autoimmune, alcoholic, and genetic causes of liver damage. In our case, no other cause of liver injury was found except the administration of LMWH. In the absence of pathognomonic biomarkers, several scores have been designed to facilitate the identification of DILI. The RUCAM score[4] is the most commonly used tool worldwide to detect the link between a drug and its potential liver injury. It is based on chronological and clinical criteria and the final score, ranging between 9 and 14 points, reflects the strength of the association of causality between the suspected drug and the liver injury (0 excludes causality; 1-2 unlikely; 5 possible, 6-8 probable, ≥ 9 highly probable). Applying the updated RUCAM system to our case, the total score was 9, labeling it as a highly probable DILI. In fact, there was a time window compatible with the occurrence of liver damage (documented 18 days after the first administration of LMWH) and a progressive decrease, until complete normalization, of the aminotransferases serum level only after nadroparin withdrawal. The presence of risk factors (age > 55 years) and concomitant administration of drugs, but without an association with the time of DILI onset, contributed to the overall score. Although alcohol consumption is considered a risk factor for liver damage, in our case the intake of alcohol does not seem sufficient to be considered an additional risk factor for liver injury. Considering the changes made to the patient's therapy, the only variation in drug administration in the previous 6 months was the addition of LMWH, which started 17 days before symptomatic hepatic injury. The serum level of liver enzymes did not improve after simvastatin withdrawal and the use of ketorolac was not followed by aminotransferases abnormalities. The role of esomeprazole and clopidogrel as culprit drugs was unlikely because there were no chronological (started several years earlier) and epidemiological (respectively less than 1% and 1-3% of cases) relations with hepatotoxicity. The possibility that other drugs taken by the patient may have acted as causative agents was also unlikely. However, we cannot exclude that the combination of one of these compounds with LMWH may have contributed to the overall liver damage. Hypertransaminasemia associated to unfractionated heparin treatment is known from 1975 (Table I)[7]. Indeed, despite the different sources and criteria for defining liver injury, the occurrence of elevated aminotransferases above ULN has a prevalence of 2.3-36% with LMWH, decreasing to 5-9% when considering a limit 3 × ULN[8]. According to the mole-

Table I. Main case reports reporting on heparin-induced liver injury published in literature.

Study	No. pts	Type of heparin	Diagnosis	Associated symptoms and signs	Time of onset of hyperaminotransferasemia	Pattern of liver injury	Time of normalization of LFT after drug discontinuation
Chee et al[13]	2	LMWH (1st case: Enoxaparin; 2nd case: Fraxiparin)	1st case: pulmonary embolism 2nd case: cerebral infarction	–	4 days/5 days	1st case: Cholestati; 2nd case: Mixed	1st case: 2 mo; 2nd case: 3 mo.
Carlson et al[8]	1	LMWH (Enoxaparin)	Deep vein thrombosis	Abdominal pain	4 month	NA	18 days
Baker et al[16]	1	LMWH (Enoxaparin)	Pulmonary embolism	Nausea and vomiting	2 days	Hepatocellular	2 mo
Levinson et al[17]	1	LMWH (dalteparin)	Thrombophlebitis	Fever, nausea and jaundice	33 days	Mixed	5 mo

LFT = liver function tests.

cule, the reported elevation rate of AST and ALT > 3 × ULN is 6.1% and 5.9% for enoxaparin, 4.7% and 4.2% for dalteparin, 8.8% and 13% for tinzaparin, and from 1 to 10% for nadroparin, respectively. Girolami et al[9] reported on 274 patients with venous thromboembolism randomized to unfractionated heparin, nadroparin, and reviparin. The occurrence aminotransferases elevation > 2 ULN was 2.9%, 5.7%, and 10.3%, respectively, without statistically significant difference between the different drugs; this suggests a similar risk of liver injury for LWMH and unfractionated heparin that should never be underestimated. Overall, the potential hepatotoxicity of unfractionated heparin[10] and LMWH confirms the hypothesis of a class effect. This consideration requires caution when switching to another heparin molecule in presence of DILI. The highly variable reported prevalence of LMWH-induced DILI depends on the type of heparin, the modality of administration, and the criteria used to define aminotransferasemia. The biological mechanism is still unclear. The hypothesis of immunological hepatotoxicity seems to be unlikely because hypersensitivity reactions as eosinophilia, rash, fever, and thrombocytopenia have never been reported. Furthermore, heparin metabolism occurs through desulphation, thus the role of heparin itself as a direct hepatocellular toxin could be excluded[11]. The most plausible explanation appears to be the modification of hepatocyte membrane by the drug[8]. However, this hypothesis has been confuted by Harrill et al[12], who conducted a study of 48 healthy subjects randomized to unfractionated heparin, enoxaparin sodium and adomiparin sodium with the aim to monitor aminotransferases elevation. A higher frequency (more than 90% of patients) of serum aminotransferases elevation was reported, and the quantification of liver-specific protein biomarkers suggested that heparin may cause transient hepatocytes necrosis and activation of the innate immune-response that may contribute to liver tissue damage even after drug discontinuation. The reason why liver injury is not associated with a functional disorder is not yet clear. Indeed, the alteration of liver tests is not accompanied by liver dysfunction but, as in our case, it is not always self-limiting and may worsen, requiring drug withdrawal. In our patients, there was a marked increase in liver enzymes during the follow-up. The increase in serum aminotransferases is believed to be maximal within 7 days of therapy[13], but in our case the peak was reached 3 weeks after the first administration, with a magnitude never reported before (aminotransferases elevation higher than 48 × ULN). Finally, we would like to discuss the usefulness of liver biopsy in the diagnostic work-up of DILI, mainly to quantify liver damage, rule out other etiologies of liver injury, and for patients' follow-up. Six major histological categories of liver injury have been defined: acute hepatitis, chronic hepatitis, acute cholestasis, zonal necrosis, and cholestatic hepatitis[14]. Fibrosis, microvesicular steatosis, cholangiolar cholestasis, neutrophils, and portal venopathy are associated with severe or fatal lesions, whereas eosinophils and

granulomas (which are histological features of immunoallergic reaction) are related to less pronounced damage and a better prognosis. Previous case reports of LMWH-related DILI reported a preserved acinar architecture, ballooning degeneration with cytoplasmic swelling and clearing, mainly in the acinar zone 3 and focally also in zone 2, and the presence of scattered foci of hepatocellular necrosis without any histological features of cholestasis[13]. In our case, liver histology was characterized by the presence of several ground glass hepatocytes, with cytoplasmic inclusions, due to fibrinogen storage. On hematoxylin and eosin stained sections, these cells closely resembled ground glass hepatocytes described in other conditions (Figure 2)[15], including hepatitis B, drug-induced liver damage, type IV glycogenosis, and endoplasmic reticulum storage disease. The mechanism of their formation remains unknown.

Conclusions

The aim of this clinical case report is to contribute to DILI reports, helping clinicians to recognize an often-underestimated condition, providing more data to characterize the clinical, laboratory, and histopathological pattern of heparin-induced liver injury. The hepatotoxic effect of heparin anticoagulant treatment is usually benign and reversible, but in some cases it is not self-limiting and may lead to acute hepatitis. Our case of DILI due to nadroparin administration was unusual as regards the entity and the persistence of serum aminotransferases elevation, but demonstrates that severe liver injury can also occur. The high frequency of this condition should always be taken into account by clinicians in order to make a prompt diagnosis and avoid a useless and aggressive diagnostic work-up. We suggest that the monitoring of the liver function tests could be considered during heparin treatment, and when a persistent and remarkable LWMH induced liver injury occurs, it is recommended to switch to another class of anticoagulants.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Declaration of Funding Interests
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