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by Wolfgang Miesbach and Vincenzo Cardinale

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Beyond transaminitis: liver biopsy redefines human gene therapy hepatotoxicity

Wolfgang Miesbach[1] and Vincenzo Cardinale[2,3,4]

1) Department of Coagulation Disorders/Haemophilia Centre, Medical Clinic 2, University Hospital Frankfurt, Frankfurt, Germany
2) Department of Internal Medicine, Division of Gastroenterology, Saint Louis University School of Medicine-SSM SLUCare, SLU Hospital, 1008 S. Spring St. Louis, MO 63110, USA
3) Department of Translational and Precision Medicine, Sapienza University of Rome, Viale dell'Università 37, Rome 00185
4) Department of Medicine, Harvard Medical School, 99 Brookline Ave, Boston, MA 02215, USA

Corresponding Author
Vincenzo Cardinale MD, PhD
Department of Internal Medicine, Division of Gastroenterology, Saint Louis University School of Medicine-SSM SLUCare, SLU Hospital, 1008 S. Spring St. Louis, MO 63110, USA. vincenzo.cardinale@health.slu.edu. Phone: +1 413-210-9259
Department of Translational and Precision Medicine, Sapienza University of Rome, Viale dell'Università 37, Rome 00185. vincenzo.cardinale@uniroma1.it. Phone: +39 3495601492

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In this issue of Haematologica, La Mura et al. (1) present the first detailed histopathological, ultrastructural, and immunophenotypic characterization of liver injury following valoctocogene roxaparvovec gene therapy for [severe hemophilia A] (2). By performing transjugular liver biopsies in two of five treated patients who developed clinically ALT elevations with loss of transgene expression, the authors provide biopsy-proven evidence of genuine immune-mediated hepatitis — advancing the field on three critical fronts: mechanistic understanding, histological classification, and clinical management.

Until now, the mechanistic framework of AAV-associated liver injury has rested almost entirely on preclinical models and *in vitro* human cell systems. Two innate immune triggers identified within the first week post-infusion: - TLR2 recognition of capsid proteins on Kupffer cells activating NF-κB (3) and endosomal TLR9 sensing of unmethylated CpG motifs in the vector genome by plasmacytoid dendritic cells (pDCs). As reviewed by Cao et al. (4), these pathways are likely redundant, ultimately priming capsid-specific CD8+ T cells. In the adaptive phase (4–8 weeks), these T cells target transduced hepatocytes presenting capsid-derived peptides on MHC class I — a mechanism characterized in murine and in nonhuman primate liver (NHP), but never previously confirmed in human liver tissue during active injury.

This sequence mirrors idiosyncratic DILI, where reactive metabolites — here, capsid-derived adducts and CpG-containing vector DNA cause hepatocyte stress with release of damage-associated molecular patterns (DAMPs), activating inflammasomes in antigen-presenting cells. The dominant hepatic immune response is tolerance; clinically significant adaptive immune injury ensues only when this tolerance fails.

The biopsy findings of La Mura et al. provide the first human confirmation: CD8+ T-cell predominant interface hepatitis with plasma cell infiltration, high cytotoxic and low regulatory T-cell activity, occurring at the expected immune window. Critically, the interface hepatitis is mild in both index cases. Moderate-to-severe interface hepatitis is the hallmark of idiopathic AIH, while milder inflammation favors DILI (5). The 2023 joint IAIHG/EASL DILI Consortium expert consensus (6) defined DI-ALH as an injury that may be indistinguishable from idiopathic AIH, but rarely requires long-term immunosuppression. The corticosteroid-responsive course described by La Mura et al., without need for prolonged immunosuppression aligns with this definition and positions these biopsies within the DI-ALH spectrum.

The Fong (7) and La Mura (1) biopsies together bracket the two ends of the hepatic response. Fong et al. sampled clinically stable patients years after dosing, finding transduced hepatocytes carrying full-length circular episomes — the DNA species that mediates durable expression (7, 8) — without significant inflammation: the tolerized steady state. La Mura et al. captured tolerance failure during peak ALT, with cytotoxic T-cell activity *and* endoplasmic reticulum stress in the same biopsies. This contrast clarifies why an immune attack on transduced hepatocytes threatens efficacy: CD8+ T cells destroy precisely the episome-bearing cells responsible for long-term FVIII production. A second novel observation strengthens the case against the term "transaminitis". Beyond immune activation, La Mura et al. documented marked endoplasmatic reticulum proliferation on electron microscopy with elevated with elevated BiP, XBP1s, and CHOP, indicating unfolded protein response activation, Immune-mediated injury and

intracellular ER stress from high transgene load thus coexist during the acute event. "Transaminitis" — ubiquitous in gene therapy literature, including the 2025 WFH Guidelines (9) — collapses at least three distinct processes (dose-dependent hepatocyte stress, innate activation, and adaptive immune hepatitis), each with different therapeutic implications.

The La Mura cohort illustrates why this matters (1). Of the five treated patients, two developed ALT elevations exceeding 1.5-fold above baseline but remaining within the normal range. Under current protocols, these patients would have qualified for corticosteroids. The hepatology team judged that high-dose immunosuppression for transaminase still within normal limits was not warranted. Authors argued that they considered liver biopsies to rule-out immune-mediated hepatitis, but the patients refused liver biopsy. This directly challenges the assumption that any ALT rise above 1.5-fold baseline mandates immunosuppression. Rather than reflexively treating a laboratory threshold, timely immunosuppression should target the window of tolerance failure, which liver biopsy can identify and distinguish from the self-limited innate response. These findings must be read with appropriate caution: they derive from two biopsy-proven cases at a single center, and the authors rightly frame their proposed algorithm as hypothesis-generating. Prospective validation is needed before it displaces current WFH and ISTH guidance. Nonetheless, the direction is compelling. CpG-depleted vector designs that reduce immunogenic load while maintaining efficacy (4) may ultimately lessen the problem at its source.

These findings reinforce the call — first articulated in our 2023 commentary (10) — for systematic collaboration between haematologists and hepatologists at all stages of gene therapy. La Mura et al. demonstrates that hepatological expertise and multidisciplinary collaboration can identify true immune-mediated hepatitis when it occurs and prevent unnecessary immunosuppression when it does not. Prospective biopsy protocols, standardized histological reporting using the DI-ALH framework, and NIT-guided long-term surveillance should be priorities for the field. La Mura et al. have shown us the path forward at the edge of hepatology, hematology, immunology and gene therapy.

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