Transplant Immunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Review

Non invasive diagnosis of acute cellular rejection after liver transplantation – Current opinion

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ARTICLE INFO

Keywords:
Acute cellular rejection
Biomarkers
Noninvasive diagnosis
mRNA
Cytokines

1. Introduction

Acute cellular rejection [ACR] or T-cell mediated rejection, is a relatively common phenomenon in liver transplantation, occurring in 20–60% of patients, despite the liver being considered immunologically privileged [1,2]. ACR is a complex immunologically mediated vascular phenomenon which targets the biliary tree, engaging a wide range of cells, cytokines and receptors in the process [Fig. 1]. Most episodes occur within the first year and there is an early peak in the first 6 weeks after transplantation [1]. Histologically, many criteria such as the Pittsburgh, Minnesota, European and the Royal Free Hospital criteria that were in use in the 80s and 90s, have been replaced by the Banff criteria for the diagnosis and grading of ACR [3,4]. The Banff schema scores 3 aspects of histological alterations that are known to occur in ACR in the liver, namely, mixed portal inflammation, bile duct damage and venous endothelitis, on a scale of 0–3 each. The composite score, the rejection activity index [RAI] grades ACR into mild [score: 3–4]; moderate [score:5–6] and severe [score:7–9]. Severe ACR and some elements of the Banff schema correlate with graft outcomes and response to steroids [5].

Although a few decades ago, timed liver biopsies between 7 and 10 days post liver transplant ['protocol biopsies'] and follow up biopsies after a steroid pulse to assess response were the norm in most liver transplant centres, this practice has changed to an 'on demand' biopsy based on clinical suspicion of ACR. Clinical signs such as malaise, low grade pyrexia and mild abdominal discomfort though common, are nonspecific in the post-transplant setting. In practice, the diagnostic algorithm usually starts with a clinical suspicion of ACR in the face of raised transaminases [AST/ALT], raised 'enzymes of cholestasis' [Alkaline phosphatase/ Gamma glutamyl transferase] and or raised bilirubin, which is otherwise unexplained at that time point in the clinical pathway. This biochemical manifestation of graft dysfunction is also nonspecific. The causes which need to be ruled out, before the diagnosis of ACR include, vascular pathology [hepatic artery thrombosis; portal vein thrombosis; outflow obstruction]; biliary pathology [leak; stricture; cholangitis; intrahepatic cholestasis]; sepsis; cytomegalovirus infection and drug induced liver injury, to name a few.

Currently the gold standard in the diagnosis of ACR is a percutaneous biopsy of the liver, which has a risk of morbidity and may suffer from sampling issues. A non-invasive tool with high sensitivity and specificity would be valuable. A clear distinction should be made between risk factors or associations with ACR which predict a higher than usual future probability of ACR [6–14] and markers of ACR which have a diagnostic value during or just before the event. While these may be useful to risk stratify and probably modify immunosuppressive strategies, these in general are not practically useful to diagnose ACR, and hence will not be discussed further [Table 1]. The current effort is to review the various non-invasive tools available in the diagnosis of ACR [Table 2].

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https://doi.org/10.1016/j.trim.2018.02.002
Received 31 October 2017; Received in revised form 31 January 2018; Accepted 5 February 2018

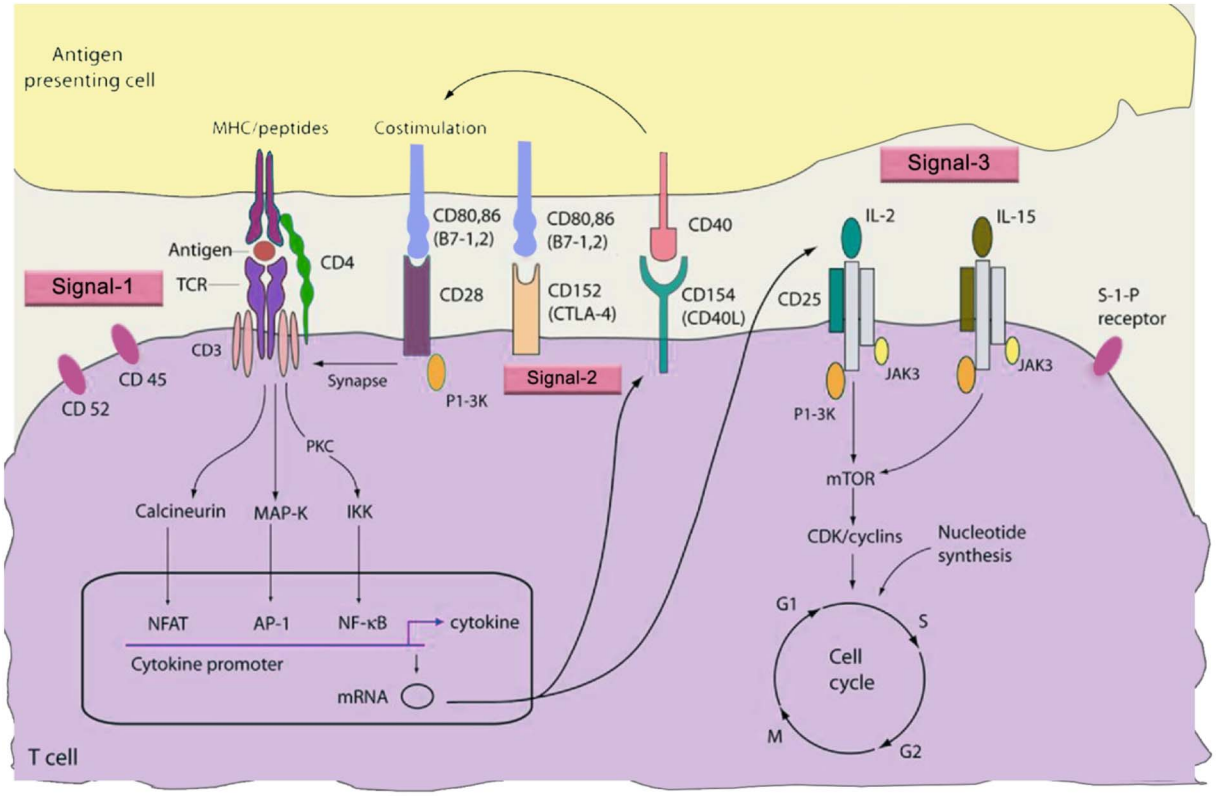

Fig. 1. Pathophysiology of acute cellular rejection.
Multiple signaling pathways of T cell proliferation: The first signal is activated when major histocampatibility complex (MHC) bound antigen is presented to T cell via T-cell receptor (TCR) by the antigen presenting cell (APC). Engagement of CD80 (B7-1) and CD86 (B7-2) on the APC, with CD28 receptor of T-cell leads to costimulation (Signal 2). Various signal transduction pathways (calcineurin, RAS- [MAP-K] pathway, and the nuclear factor-kappa B [NF-kB] pathway) lead to the expression of many cytokines like interleukin-2 and interleukin-15. These cytokines then activate the "mammalian target of rapamycin" (mTOR) pathway which is the final pathway for T-cell proliferation (signal 3).
TCR - T cell receptor; PKC - protein kinase C; MAP-K - mitogen-activated protein kinase; IKK – I kappa B kinase; NFAT - nuclear factor of activated T cells; AP-1 - activating protein 1; mRNA - messenger ribonucleic acid; JAK3 - Janus kinase 3; S-1-P - sphingosine-1-phosphate; PI 3-K - phosphoinositide 3 kinase; CDK - cyclins-dependent protein kinase. (Modified from Sharon A. Hunt: The changing face of heart transplantation. J Am Coll Cardiol, Vol. 52, No. 8, 2008 August 19, 2008:587–98) [112].

Table 1
Perioperative predictive markers, for an increased risk of future ACR.

Parameter	Features	Reference
Pre-operative AEC > 130/micl	Sensitivity 72%; specificity 66% NPV 82% for ACR.	Dollinger [6]
Transaminases > 20 times ULN	On post op day 1 is associated with a later ACR	Hickman, Naik [7,8]
Serum IFNϒ$^{+}$	> 4.5 iu/ml in first week predicts future rejection	Sood [9]
Pre transplant TNF alpha	Predicts post transplant ACR	Bathgate [10]
Intracellular IL-2 in CD8^{+} T cells	Overexpression pretransplant predicts ACR	Boleslawski [11]
Low CD44 + high CXCL-9 in serum	On post op day 1 predicts future ACR	Raschzok [12]
Proportion of CD8^{+} IFNϒ$^{+}$ Tcells	If > 56% preop, predicts ACR; sensitivity 75%; specificity 82%.	Millan [13]
Gene expression profiling	Identifies graft tolerance in LT. Some evidence for value in ACR prediction in renal transplant. Potential in LT as yet unproven.	Heidt [14]

AEC- Absolute eosinophil count; NPV- Negative predictive value; ACR- Acute cellular rejection; ULN- Upper limit of normal; INF- Interferon; TNF- Tumour necrosis factor; IL- Interleukin; CD- Cluster of differentiation; LT- Liver transplant.

2. Markers in blood

2.1. Liver biochemistry

The liver function test panel [LFT] is non-specific and has a low sensitivity in diagnosing ACR. Correlation with simultaneous liver biopsy findings is poor [15–17]. However, certain trends are apparent and a combination of trends of the candidates in the panel may help guide clinical judgements and decision making [7,8,18]. Bilirubin and transaminases do show a rising trend during ACR, but the magnitude and rate of change is variable and no cut-offs had been validated until recently. In a deceased donor liver transplantation [DDLT] cohort, a total bilirubin of > 4 mg%, a rising bilirubin in the previous 4 days and an absolute eosinophil count [AEC] of > 100/μl were markers of moderate-severe ACR on biopsy [19]. Patients who later developed ACR, tended to have a day 1 transaminase levels which were about 20 times upper limit of normal [ULN] as compared to non-rejectors who had them in the range of 4–12 times ULN [7,8,20].

In general, patients with ACR will show a rising trend in bilirubin before or during the episode, but a proportion will show a reducing trend in the days preceding the biopsy, even before commencement of pulse steroids [7]. Total bilirubin level, hence, is not a reliable marker of ACR. However, fractional analysis of bilirubin may be useful. Direct bilirubin exists in a protein bound form named Delta bilirubin [Bd] and a non-protein bound conjugated form [Bc]. Within the normal range, direct bilirubin is mostly in the delta form, while in conditions of elevated direct bilirubin, the Bc form rises significantly. In a study of 80 patients of whom 18 developed ACR, an elevated Bc of > 0.1 mg% detected ACR with a sensitivity of 78% specificity of 77%, PPV of 50% and NPV of 92% [21]. Similarly, in a study of 51 patients, a decrease in Bd [< 30% of total bilirubin] and a reciprocal increase in Bc [> 50% of total bilirubin] has been shown to be an accurate marker of ACR with sensitivity and specificity over 92% and NPV over 98% [22]. Muraca et al. found that serum esterified bilirubin [a conjugated form of