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Discordant molecular tests for acute Graft-Versus-Host Disease after liver transplant: FISHing for the proper diagnosis

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Abstract

We report cutaneous fluorescent in-situ hybridization (FISH) analysis as a useful test for earlier diagnostic confirmation in a case of acute graft-versus-host disease (GVHD) in a sex-mismatched liver transplant patient compared to the gold standard peripheral blood short tandem repeat chimerism analysis.

Keywords: immunopathology, diagnosis, skin diseases in transplant recipients

Case Synopsis

A woman in her 60's with a history of alcoholic cirrhosis underwent orthotopic liver transplantation from a male donor, followed by induction immunosuppression with basiliximab and prednisone, and maintenance with mycophenolate and tacrolimus. On post-operative day 34, she presented with altered mental status and a new rash. Laboratory tests were significant for hemoglobin 7.5g/dL (11.7-15.5 ref), white blood cells 2.6K/µL (4.5-11 ref), platelets 422K/µL (150-400 ref), potassium 2.9mmol/L (3.5-5.0 ref), and serum creatinine 4.13mg/dL (0.5-1.2 ref). Dusky and violaceous, scaly macules and papules predominated on the upper trunk and extremities (**Figure 1**). Histology revealed a mild interface dermatitis with scattered necrotic keratinocytes in the epidermis and papillary dermal

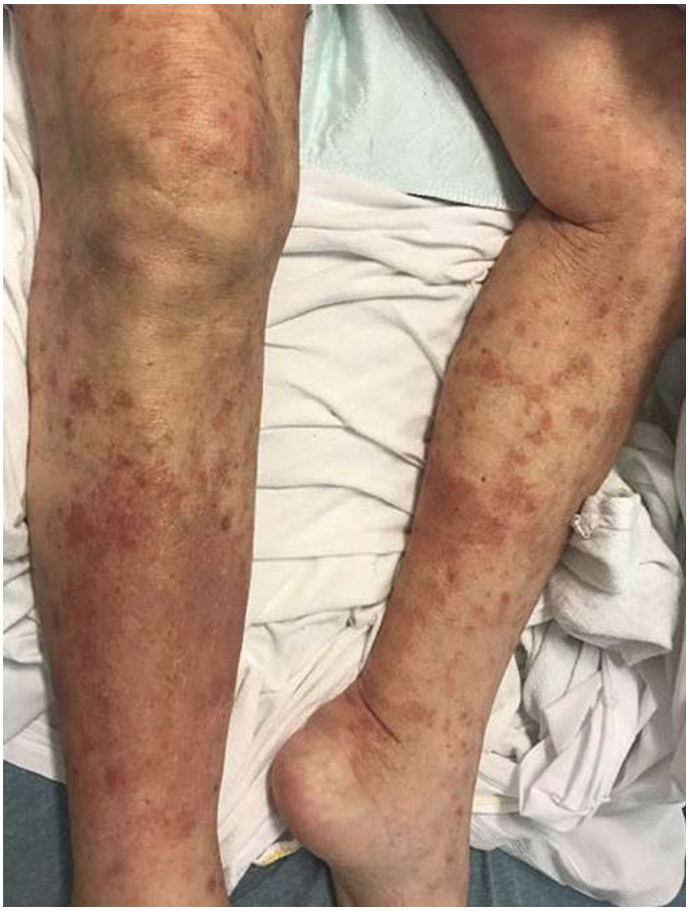

Figure 1. *Cutaneous lesions on initial presentation — erythematous to dusky, slightly scaly macules coalescing into patches on the patient's lower extremities.*

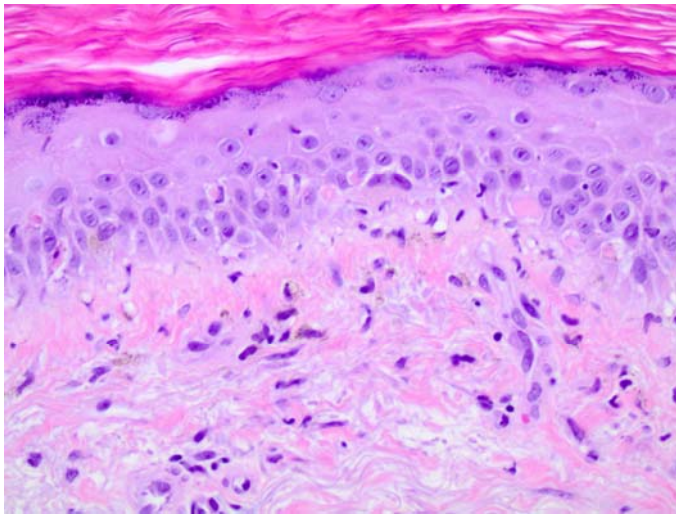

Figure 2. *Histological findings from skin biopsy — mild interface dermatitis with scattered necrotic keratinocytes in the epidermis and papillary dermal pigment incontinence. H&E, 20×.*

pigment incontinence (**Figure 2**). The patient was clinically diagnosed with cutaneous acute GVHD. However, peripheral blood chimerism studies by polymerase chain reaction (PCR) failed to reveal donor cells in the peripheral blood and her cutaneous lesions improved over the next several days.

On post-operative day 63, without clearance, the patient had ongoing gastrointestinal symptoms and progressive pancytopenia (**Table 1**); her post-operative day 34 skin biopsy was sent for fluorescent in situ hybridization (FISH) analysis. Enumeration of cells with leukocyte morphology showed 79% XY and 21% XX, confirming the diagnosis of cutaneous GVHD, despite continued negative peripheral chimerism by PCR (**Figure 3**). On post-operative day 87, PCR of purified T cells demonstrated donor chimerism and FISH analysis of bone marrow aspirate showed 14.2% XY donor cells. The patient transitioned to comfort care and died from pancytopenia on POD 98.

Case Discussion

Acute GVHD is a rare complication of orthotopic liver transplantation with mortality in 80% of cases [1]. Early diagnosis is critical in preventing poor outcomes. Detection of peripheral blood macrochimerism (>1% donor HLA alleles in recipient blood) is generally used for diagnostic confirmation of acute GVHD [2]. Chimerism studies utilize PCR to amplify short tandem repeats from post-transplant genomic DNA and compare those amplicon sizes to those from recipient and donor organ samples. In orthotopic liver transplantation, transient donor T cell chimerism occurs in more than 50% of patients immediately post-surgery and lasts for 1-3 weeks, peaking at an average of 5% donor T cell proportion. Persistent blood chimerism usually indicates acute GVHD but can also indicate immune tolerance [3].

In rare situations, patients can present with signs and symptoms of acute GVHD, but have negative peripheral chimerisms [1, 4, 5]. This can delay diagnosis and reduce the likelihood of receiving treatment. FISH analysis of affected tissue has been shown to confirm diagnosis of GVHD in sex-mismatched orthotopic liver transplantation patients [6, 7] and can be used as an additional test when the diagnosis is still in question. In our case, a negative blood chimerism study was followed by FISH analysis on the initial skin biopsy that showed the presence of XY donor cells in our female patient, confirming GVHD. The findings in our case support findings from similar cases that have reported earlier diagnosis of GVHD via analysis of affected tissue compared with peripheral chimerism studies [1, 8].

Table 1. *Post operative cell counts.*

Post-Op Day	Hemoglobin	White Blood Cells	Platelets
34	6.9	5.3	432
39	8.6	7.7	189
44	6.9	2.7	84
49	7.1	0.6	86
54	7.9	3.4	160
59	6.5	3.1	274
64	8	2.2	177
69	7.2	1.3	88
74	8.4	6.6	28
79	7.4	3.78	40
84	7.5	0.91	52
89	7.3	0.38	44
94	7.8	0.3	32

The optimal timing for blood chimerism testing to most effectively diagnose acute GVHD is not fully established and can be negative early in the disease course, leading clinicians away from a diagnosis of GVHD. If index of suspicion is high in a sex-mismatched organ transplant, we suggest using FISH to analyze the affected tissue.

Conclusion

Our report identifies a case of a patient with a delayed formal diagnosis of acute GVHD owing to reliance on peripheral blood chimerism analysis. As solid-organ transplant-associated graft-versus-host disease is nearly universally fatal, early diagnosis potentially can allow for intervention and consideration of an allogeneic stem cell transplant [9]. Dermatologists should be aware of the availability of FISH analysis on skin biopsy specimens to facilitate the diagnosis of acute GVHD in solid-organ transplant patients with high clinical suspicion for acute GVHD despite negative peripheral blood chimerism studies.

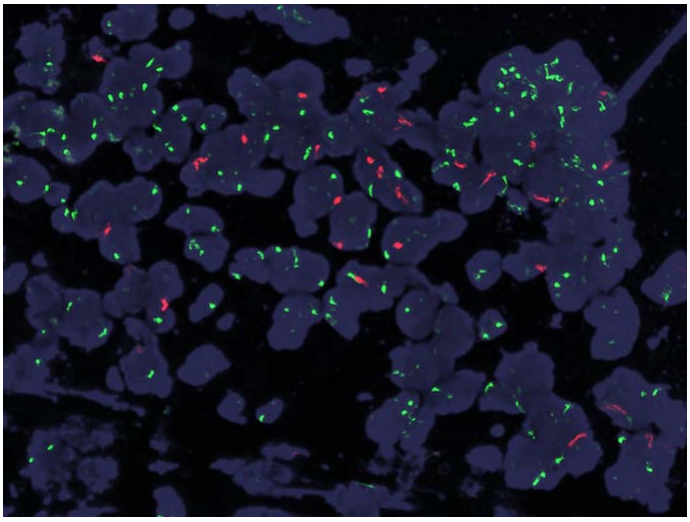

Figure 3. *XY fluorescent in-situ hybridization analysis of the skin biopsy (CEP X Spectrum Green and CEP Y Spectrum Orange, Abbott Molecular/Vysis, Abbott Park, IL) – numerous small cells at the dermal-epidermal junction, mostly representing lymphocytes, show 1-red/1-green XY donor signal pattern, 600×.*

Potential conflicts of interest

The authors declare no conflicts of interests.

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