Chimerism testing for postmortem evaluation of Solid Organ and Transfusion associated Graft-vs-Host disease

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BACKGROUND

A 66-year-old male with idiopathic pulmonary fibrosis underwent a bilateral lung transplant (LT) from a female donor. He was transfused several units of leukoreduced, but not irradiated pRBCs at the time of transplant with 1 unit coming from a female donor. The patient and their lung donor were nearly a complete HLA mismatch, sharing only C*15 and DRB3*03. Retrospective crossmatch testing was T and B cell negative with no detected DSA. The patient was readmitted 5 weeks after hospital discharge with fever, transaminitis, and a non-pruritic, erythematous, maculopapular rash. Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome was suspected and culprit medications were stopped. He then developed rapidly progressive pancytopenia which prompted a bone marrow biopsy. The biopsy revealed an XX karyotype and a subsequent skin biopsy confirmed a mixed XX/XY karyotype. Despite multiple interventions, the patient died 15 weeks after LT and the HLA laboratory was contacted to facilitate additional genetic testing postmortem. Chimerism testing was recommended due to a clinical suspicion for graft-versus-host disease (GVHD).

GVHD, most often observed following Hematopoietic Stem Cell Transplantation (HSCT), has been reported following solid organ transplantation (SOT) or transfused blood products when donor-derived lymphocytes are transferred into an immunocompromised recipient, become activated, and target recipient tissues. Both SOT and transfusion associated (TA)-GVHD carry a poor prognosis and high mortality rates.

OBJECTIVE

To determine if formalin-fixed paraffin embedded (FFPE) bone marrow (BM) specimens collected post-bilateral lung transplant can be utilized for postmortem donor chimerism in a case of suspected SOT and/or TA-GVHD

METHODS

- Chimerism testing was performed per package insert (AlloSeq HCT, CareDx) using Next Generation Sequencing (NGS) which evaluates 202 bi-allelic single nucleotide polymorphisms (SNPs).
- Data analysis was performed using AlloSeq HCT software versions 1.1.0 and 2.1.3.
- Pretransplant recipient and lung donor genotypes were established using DNA extracted from peripheral blood samples.
- Post-transplant DNA samples were extracted from FFPE bone marrow biopsies (*Quick*-DNA FFPE Miniprep, Zymo Research).
- Bone marrow biopsies were collected on 8/24/21 (sample 1) and 8/30/21 (sample 2).

RESULTS

SNPs		Allele		Recipient (Pre-Tx)					Lung Donor (Pre-Tx)					Sample 1 (Post-Tx)					Sample 2 (Post-Tx)			
Target ID		**Ref.**	**Alt.**	**Geno.**	**A**	**C**	**G**	**T**	**Geno.**	**A**	**C**	**G**	**T**	**A**	**C**	**G**	**T**	**A**	**C**	**G**	**T**
rs3098168	chr15	T	C	**T/T**	0	0	0	642	**T/C**	0	367	0	394	0	45	0	680	0	40	0	212
rs62180470	chr2	A	G	**G/G**	1	0	4503	0	**A/G**	2437	0	2577	0	76	0	1912	0	97	0	920	0
rs1449445	chr3	T	C	**T/T**	2	0	0	3062	**T/C**	0	1875	0	1812	0	88	0	1798	0	85	0	1404
rs7160372	chr14	T	C	**C/C**	0	4546	0	0	**T/C**	0	2577	0	2552	0	1794	0	92	0	708	0	49
rs623052	chr11	G	A	**A/A**	1893	0	0	0	**G/G**	0	0	1973	0	937	0	99	0	442	0	41	0
rs2121881	chr6	C	A	**C/C**	0	980	0	0	**A/A**	1519	0	0	0	68	465	0	0	20	91	0	0
rs4924176	chr15	A	T	**T/T**	1	0	0	2397	**A/A**	2983	0	0	0	70	0	0	697	23	0	0	76
rs471234	chr11	C	A	**A/A**	4980	2	1	0	**C/C**	4	5702	0	0	1961	222	1	0	1600	255	0	0
rs4353533	chr17	C	T	**T/T**	0	0	0	578	**C/C**	0	755	0	1	0	85	0	780	0	112	0	1041
rs9304650	chr19	C	T	**C/T**	0	2948	0	3584	**C/C**	0	7143	0	2	0	749	0	664	0	**163**	0	**478**
rs1201673	chr3	A	G	**A/A**	1433	0	0	0	**A/A**	1793	0	1	0	481	0	0	0	46	0	**3**	0
rs11156787	chr14	A	G	**G/G**	1	0	2832	0	**G/G**	1	0	3496	0	0	0	1083	0	**8**	0	207	0
rs2124299	chr18	T	C	**C/C**	0	1405	0	0	**C/C**	0	1863	0	1	0	850	0	**1**	0	314	0	**7**
rs6774979	chr3	A	G	**G/G**	0	0	671	0	**G/G**	0	0	916	0	0	0	721	0	**3**	0	264	0
rs1622264	chr5	A	T	**A/A**	2593	0	0	0	**A/A**	3481	0	3	0	1468	0	1	0	316	0	0	**4**
rs1494338	chr9	C	T	**T/T**	0	0	0	2018	**T/T**	0	0	0	2797	0	0	0	783	0	**3**	0	69
rs7974066	chr12	T	C	**T/T**	0	0	0	476	**T/T**	0	0	0	673	0	**8**	0	324	0	**4**	0	94
rs36158849	chr12	T	A	**T/T**	0	0	0	2264	**T/T**	0	0	0	2000	0	0	0	953	**6**	0	0	484

Table 1: Select SNPs comparing pre-transplant (Pre-Tx) recipient and lung donor genotypes to those determined for posttransplant (Post-Tx) samples collected six days apart. A single run replicate for samples 1 (collected 8/24/21) and 2 (collected 8/30/21) are shown. Bold numbers indicate the nucleotide counts at specific positions that can likely be attributed to the presence of a second donor.

Sample 1 (replicates)	%Recipient	%Lung Donor	%Donor 2
1.1	90.4	9.1	0.5
1.2	91.0	8.5	0.5
Average	**90.7**	**8.8**	**0.5**

Table 2: Percent double donor chimerism detected using DNA isolated from a preserved (FFPE) bone marrow biopsy

Sample 2 (replicates)	%Recipient	%Lung Donor	%Donor 2
2.1	84.6	9.8	5.6
2.2	86.5	7.9	5.6
2.3	84.8	10.5	4.7
Average	**85.3**	**9.4**	**5.3**

Table 3: Double donor chimerism evaluation detected an increasing percentage of a second donor (transfusion associated?) using DNA isolated from an FFPE bone marrow biopsy collected six days after sample 1

DISCUSSION

- GVHD in LT recipients is a rare occurrence.
- The risk for GVHD resulting from transfused blood products can be eliminated or significantly reduced through irradiation or leukoreduction respectively.
- Chimerism testing can provide supporting evidence for diagnosing GVHD for patients that display a high likelihood for the disorder through other clinical manifestations like those described in the background section.
- Our study has demonstrated that this technology can utilize DNA isolated from preserved sources, like FFPE, making this approach amenable for use in postmortem investigations.

CONCLUSIONS

Both SOT and TA-GVHD carry a poor prognosis with a high mortality rate, thereby requiring an early diagnosis and a high index of suspicion. Our experience supports the use of chimerism testing for the evaluation of GVHD following SOT. This approach may facilitate an earlier diagnosis and prompt intervention. Additionally, our work supports the use of chimerism testing for postmortem investigation of suspected GVHD cases using non-traditional sample sources.