CD4 Count in HIV– Brain-Dead Donors: Insight into Donor Risk Assessment for HIV+ Donors

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Background. The Human Immunodeficiency Virus (HIV) Organ Policy Equity Act allows for transplantation of organs from HIV-infected individuals (HIV+), provided it is performed under a research protocol. The safety assessment of an organ for transplantation is an essential element of the donation process. The risk for HIV-associated opportunistic infections increases as circulating CD4+ lymphocytes decrease to less than 200 cells/ μ L; however, the numbers of circulating CD4+ cells in the HIV-negative (HIV–) brain-dead donor (BDD) is not known. **Methods.** Circulating T-lymphocyte subset profiles in conventional HIV– BDD were measured in 20 BDD in a clinical laboratory. **Results.** The mean age of the BDD cohort was 48.7 years, 95% were white and 45% were women. The average body mass index was 29.2 kg/m². Cerebrovascular accident (40%) was the most prevalent cause of death. Sixteen (80%) subjects had a CD4 count $\leq$ 441 cells/ μ L (lower limit of normal) and 11 (55%) had a CD4 count less than 200 cells/ μ L; 11 (55%) subjects had a CD8 count $\leq$ 125 cells/ μ L (lower limit of normal). CD4/CD8 ratio was below normal in 3 patients (normal, 1.4–2.6). No recipient had a recognized donor-associated adverse event. **Conclusions.** Absolute numbers of CD4 and CD8 T-lymphocytes are commonly reduced after brain death in HIV– individuals. Thus, CD4 absolute numbers are an inconsistent metric for assessing organ donor risk, irrespective of HIV status.

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Organ transplantation is a successful therapy for many human immunodeficiency virus (HIV)+ individuals with end-stage renal and liver disease.^{1–4} Furthermore, a recent study from South Africa demonstrated that HIV+ kidney recipients receiving transplants from HIV+ deceased donors had a 74% overall patient survival and 84% death-censored 5-year graft survival. All recipients have maintained an undetectable plasma viral load with effective antiretroviral therapy.⁵

In an effort to expand the availability of transplantable organs for HIV+ patients, the US Congress passed the HIV Organ Policy Equity Act (HOPE) Act (42 U.S.C. § 274f-5(b)) in November 2013, mandating a revision to the National Organ Transplant Act of 1988 prohibition of transplanting organs from HIV+ donors. An important goal of the HOPE Act is to generate evidence-based, research-driven data to put forth

criteria that would facilitate the feasibility of HIV-to-HIV transplantation in the United States. It has been estimated that HIV-to-HIV transplantation could reduce the transplant waitlist by approximately 3.2% in the most conservative estimates, affecting both HIV+ and HIV– candidates.⁶

One of the primary concerns of using any donor organ is the risk of disease transmission through its transplantation. A CD4 count less than 200 cells/ μ L has historically been associated with late stage HIV disease and an increased risk for opportunistic infections (OI).^{7–10} Although the HOPE Act Research Criteria does not explicitly set a CD4 count requirement, it urges transplant teams to assess donors with CD4 counts 200 cells/ μ L or less with special caution for potential disease transmission.¹¹ At present, it is abstruse whether a CD4 count of 200 cells/ μ L or less has any additive importance upon the risk assessment for a potential organ recipient. With no available information on CD4 counts in the deceased donor population (regardless of HIV-status), the objective of this study was to screen HIV– brain-dead donor (BDD) as a comparator for future HIV+ donors.

MATERIALS AND METHODS

Approval to evaluate donors was obtained from the Institutional Review Board of the LifeSource Upper Midwest Organ Procurement Organization Research Committee and Director of Quality and Regulatory Affairs. LifeSource Upper Midwest Organ Procurement Organization typically coordinates the care of 170 donors per year with approximately 20% of donors donating after cardiocirculatory arrest. BDD for whom it was logistically feasible to collect and analyze blood samples within the required time frame (24 hours) by the immunologic assay were considered for inclusion into this study.

All donors were managed according to standardized organizational guidelines that have previously been reported¹²

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TABLE 1.
Demographic characteristics of BDD

Age, y	48.7
Sex, female (%)	9 (45%)
Ethnicity, white (%)	19 (95%)
BMI, kg/m ²	29.2
Blood type	
A	6 (30%)
B	2 (10%)
AB	2 (10%)
O	10 (50%)
Cause of death	
Cerebrovascular accident	8 (40%)
Anoxia	7 (35%)
Head trauma	4 (20%)
Medical history	
Hypertension	8 (40%)
Neurologic disease	2 (10%)
Diabetes mellitus	1 (5%)
Tobacco use	9 (45%)
Heavy alcohol consumption	10 (50%)
IV drug use	2 (10%)
Public Health Service high risk	4 (20%)

BMI, body mass index; IV, intravenous.

including: the use of intravenous fluids and inotropic agents to maintain adequate organ perfusion, ventilator management to achieve adequate oxygenation, and hormone (levothyroxine, insulin, methylprednisolone) and electrolyte repletion therapy to interdict deficiencies associated with death.

Donor demographic and clinical data were collected from United Network for Organ Sharing donor management records, including: age; cause of death; minutes of cardiac down time; initial and final vasopressor requirements; initial and final central venous pressure; smoking history; history of hypertension and diabetes mellitus; history of previous chest surgery; HIV and hepatitis B virus or hepatitis C virus (HCV) status; terminal blood tests; and classification of high-risk donor status, per Public Health Service (PHS) guidelines.

Blood samples were collected with routine donor management, within an average 32 hours (range, 12-72 hours) of brain death pronouncement but prior to organ procurement. Appropriate samples were analyzed at the University of Minnesota Laboratories using the BD Multitest 6-color TBNK immunofluorescence kit (BD Biosciences, San Jose, CA). The assay utilizes a 6-color direct immunofluorescence reagent for use with BD FACSCanto and BD FACSCanto II flow cytometers (BD Biosciences) to identify and determine the absolute number and percentages of the CD4 and CD8 subpopulations of T cells in peripheral blood. The immunofluorescent agents used in the assay are the following: FITC-labeled CD3, clone SK7; PE-Cy7-labeled CD4, clone SK3; and APC-Cy7-labeled CD8, clone SK1. The T-lymphocyte subset profile is reported as absolute specific T-lymphocyte count, percentage of all T-lymphocytes, and ratio of CD4 and CD8 subsets.

RESULTS

Blood samples were collected from 20 BDD between September 1, 2015, and April 5, 2016. The demographic

characteristics are listed in Table 1. The median age was 48.7 years (26-69 years). The majority of donors were white and 9 (45%) were female. The average body mass index was 29.2 kg/m². The most prevalent blood type was O (50%).

The most common cause of death was cerebrovascular accidents (CVA) (40%), followed by anoxia (35%) and head trauma (20%). On univariate analysis, there seemed to be no correlation between COD (ie, CVA vs non-CVA) and CD4 or CD8 count. Of note, 40% of subjects had an antecedent history of hypertension at the time of death, and 45% were smokers, 50% were recorded as heavy alcohol consumers, 10% were intravenous drug users, and 20% were considered increased-risk donors according to PHS criteria.

The clinical characteristics of the donors are listed in Table 2. At the time of donation, all were on low-dose vasopressors and vasopressin (or an alternative analog) per LifeSource Upper Midwest Organ Procurement Organization policy: 55% on diuretics, 95% on steroids, 90% on thyroid supplementation, 95% on insulin, and 30% on antihypertensives. At the time of procurement, the mean white blood cell count was 14 000 (range, 6.6-27) cells/μL and the mean hematocrit was 30.6 (22.4-41.5). The terminal mean creatinine was 1.4 (0.5-4.5) mg/dL and the mean INR was 1.2 (1.0-1.7). All donors were anti-HBcAB, anti-HCV, and anti-HIV negative; 30% had anti-CMV and 90% EBV IgG-positive antibodies. All were HCV and HIV nucleic acid testing negative.

The absolute CD4 count, CD4 %, CD8 count, CD8%, and the CD4/CD8 ratio of our BDD cohort are shown in Table 3 and Figure 1. Sixteen (80%) donors had a CD4 count below the lower limit of normal in healthy subjects per the assay used (404 cells/μL); and 11 donors (65%) had a CD4 count less than 200 cells/μL. Three (15%) patients had an abnormally low CD4 percentage. Eleven (55%) patients had a

TABLE 2.
Clinical characteristics of BDD

Predonation	
Vasopressors	20 (100%)
Diuretics	11 (55%)
Steroids	19 (95%)
Thyroid supplementation	18 (90%)
Insulin	19 (95%)
Anti-hypertensives	6 (30%)
Desmopressin	7 (35%)
Vasopressin	13 (65%)
Terminal blood work	
WBC, cells/μL	14 (6.6-27)
Hematocrit	30.6 (22.4-41.5)
Creatinine, mg/dL	1.4 (0.5-4.5)
AST, U/dL	108 (14-497)
ALT, U/dL	128 (8-848)
INR	1.2 (1.0-1.7)
Serology	
Anti-HBcAB	0
Anti-HCV	0
Anti-HIV	0
Anti-CMV	6 (30%)
EBV IgG	18 (90%)

ALT, alanine aminotransferase; AST, aminotransferase.

TABLE 3.**T cell subset profile**

Patient	CD4, absolute	CD4, %	CD8, absolute	CD8, %	CD4:CD8
1	110	49	58	26	1.9
2	117	27	49	12	2.4
3	124	23	67	12	1.9
4	125	43	40	14	3.1
5	132	39	47	14	2.8
6	136	36	79	21	1.7
7	144	46	49	16	2.9
8	151	43	20	6	7.6
9	152	37	26	6	5.8
10	153	30	17	3	9.0
11	166	31	125	24	1.3
12	253	26	203	21	1.2
13	318	39	229	28	1.4
14	365	37	154	16	2.4
15	402	44	225	25	1.8
16	403	35	428	38	0.9
17	436	40	296	27	1.5
18	553	55	128	13	4.3
19	613	51	225	19	2.7
20	1717	52	502	15	3.4

CD8 count at or below the lower limit of normal for healthy subjects (125 cells/ μ L) per the assay used. The average CD4/CD8 ratio was 3.0 but it ranged from 0.9 to 9.0 (normal, 1.4-2.6) with 12 (60%) subjects exhibiting an abnormal CD4/CD8 ratio.

From our cohort of 20 HIV- BDD, 68 organs were transplanted (6 hearts, 4 lungs, 16 livers, 4 pancreases, 38 kidneys). No organ recipient developed a recognized clinically relevant infectious or malignant disease transmission after organ transplantation.

DISCUSSION

HIV+ patients appear to be disproportionately affected by the shortage of organs for transplantation.^{13,14} The intent of the HOPE Act is to safely expand the numbers of organs that are available to HIV+ individuals with organ failure. An underlying necessity for organ donation is the rigorous evaluation for risk of disease transmission from the transplant event. Diminished numbers of CD4 T cells in HIV+ individuals

is a known harbinger for OI, and thus, measurement of CD4 count is a recommended component of the HIV+ donor risk assessment. A recent report from Switzerland of successful HIV-to-HIV liver transplantation noted that the donor CD4 count at the time of donation was 298 cells/ mm^3 .^{3,15} Although the South African report⁵ did not mention deceased donor CD4 counts, the primary author notes that CD4 counts less than 200 cells/ μ L were common among donors (personal communication, Elmi Muller, 20 May 2016). Neither report had a comparison of CD4 cell counts in conventionally managed, HIV-, neurologically deceased organ donors, nor could any relevant references in the current transplantation literature be identified.

In this study, the majority of routine, HIV- deceased organ donors had CD4 less than 200 cells/ μ L. In addition, there were concomitant perturbations in other lymphocyte populations in this deceased donor cohort before organ retrieval. Although it is possible that conventional strategies of deceased donor management (vasopressors, hormone replacement, diuretics) alter circulating CD4 numbers and CD4/CD8 ratio, there may also be a confounding effect of central nervous system injury. Immune dysfunction has been demonstrated after central nervous system injury in HIV- patients and thus could be one of many confounders in these findings.¹⁶⁻¹⁸ Furthermore, steroid therapy—a routine component of deceased donor management—has also been shown to reduce T cell subpopulations in a variety of patient cohorts¹⁹⁻²¹; however, the effect of steroids on CD4 count has never been systematically evaluated in brain-dead patients.

Physiologic variability of CD4 count and CD4 percentage in HIV+ and HIV- subjects^{22,23} exists normally. CD4 numbers are influenced by race, aging, and even the environment,²⁴⁻²⁷ and variations have been found between different methodologies used to estimate CD4 count or percentage.^{28,29} Furthermore, the CD4/CD8 ratio tends to increase with age and an inversion of the CD4/CD8 ratio is a marker for frailty and chronic viral infections.^{30,31} In HIV+ patients, a low CD4/CD8 ratio is commonly observed in advanced disease; and effective viral suppression is associated with an inversion of the CD4/CD8 ratio.^{32,33} These factors point out limitations associated with an isolated CD4 assay in the risk assessment of either HIV- or HIV+ deceased donors. Although the numbers of deceased donors in this series was too small to permit subpopulation analyses, what is striking from our study is that absolute CD4 numbers are often less than 200 cells/ μ L with an abnormal CD4/CD8 ratio in over half

FIGURE 1. Absolute CD4 count (A), CD8 count (B), and CD4:CD8 ratio (C) in HIV-negative brain dead donors. The red line indicates 200 CD4 cells/ μ L; the blue lines indicate the normal range (absolute CD4 count: 441-2156 cells/ μ L; absolute CD8 count: 125-1312 cells/ μ L; CD4:CD8 ratio: 1.4-2.6) in normal human subjects.

of the donors (3 lower, 9 higher). Although these findings raise many more questions than we are able to answer in our small case series, it will be difficult to discount low CD4 numbers in a HIV+ BDD.

There are 2 identifiable disease transmission issues uniquely associated with the use of organs from HIV+ donors; first the transmission of a drug-resistant HIV strain and second, transmission of OI associated with advanced HIV disease. Absolute CD4 numbers will be unlikely to help in the prediction of drug resistance or OI due to the wide fluctuation seen in HIV- donors and even CD4/CD8 ratios appear to vary in HIV- deceased donation. Accordingly, what is the optimal predonation approach to estimate the risk in the HIV+ donor evaluation process? Barring the development of new tests for detection of OI, it will be necessary to use more conventional tools. Examples include the presence of prior-treated OI, viral load, development of antiviral resistance and changing in antiretroviral regimens, predominant HIV subtype, trend in antemortem CD4 count, or coinfection with hepatitis B virus or HCV. Realistically, this type of information is unlikely to be readily available through third party historians during donor assessment and access to medical records may prove difficult during the donor evaluation process. However, it is important that the evaluation of the HIV+ organ donor attempts to capture the extent and severity of a potential donor's HIV- disease through a comprehensive assessment of the aforementioned parameters. A history of antiviral resistance is extremely important to document for it can serve as an indication to exercise heightened surveillance. Thus, it is suspected that viral load and a history of antiviral resistance would be considerably more important in the HIV+ organ donor evaluation than a CD4 count.

This study has limitations. First, the sample size is small, ethnically homogenous and only contains 4 PHS increased-risk donors, thus it could be argued that the findings are difficult to generalize to a broader donor pool. Second, the time interval between BD declaration and CD4 count measurement was short; however, our intent was to discern variability of CD4 counts in the routine, clinical predonation evaluation process in HIV- BDD as a practical metric to assess donor suitability and not study all variables associated with potential confounders. It would be difficult to discern the impact of time after brain death declaration as a variable for CD4 count because this study is underpowered to carry out such analysis. Third, the BDD organ evaluation process occurs in a very heterogeneous population (variable causes of brain death, medical comorbidities, age, therapies before donation and the timing of donation from precipitating injury) that could potentially alter CD4 count. However, despite these limitations, the intent of this study was to discern within a clinically relevant HIV-, deceased donor population whether CD4 numbers were consistent with those observed in the normal non-brain-dead healthy population.

We have shown that there is enough variability in CD4 numbers associated with brain death and donor management that absolute CD4 count as an exclusion criteria would be misguided. Irrespective of causality, we have demonstrated that HIV- BDD have low circulating CD4 T cells at the time of organ donation, as measured through conventional laboratory practice. Hence, we caution that CD4 numbers may be an unreliable predictor of risk for either advanced HIV

disease or for possible OI transmission after transplantation of organs from HIV+ donors. Risk assessment for OI within the HIV+ donor population will need to use variables other than absolute CD4 count. Finally, risk assessment of the individual considered for donation after death from circulatory cessation was specifically not included in this study population, and extrapolation of our findings to this group is unwise.

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