Clinical Chemistry 57:7
941–942 (2011)

Perspective

Transplantation Monitoring by Plasma DNA Sequencing

Y.M. Dennis Lo[1,2*]

Over the past 15 years, there has been increasing interest in the use of cell-free nucleic acids in plasma for molecular diagnostics, especially for cancer detection and prenatal diagnosis. In 1998, Lo et al. showed that donor-derived DNA sequences could be detected in the plasma of transplant recipients *(1)*. This group further showed that different transplanted organs—e.g., the heart, liver, and kidney—appeared to release different amounts of DNA into the plasma, probably relating to the size of the organ *(2)*. Furthermore, details in the design of the molecular assay have been shown to have important implications for detecting circulating donor-derived DNA. For example, the use of a detection target, *DYS14 (1, 3)*, which is a member of a multicopy Y chromosome–specific gene, *TSPY1*[3] (testis specific protein, Y-linked 1), has produced a higher sensitivity in detecting circulating donor-derived DNA than when the single-copy gene *SRY* (sex-determining region Y) is used *(2)*.

As cell death is generally accepted to be an important reason for the release of DNA into the plasma, Lo et al. further hypothesized that the measurement of donor-derived DNA in the plasma of transplant recipients might be used for monitoring graft rejection *(1)*. This hypothesis has subsequently been shown to be correct by a number of independent workers *(4, 5)*. The usefulness of this strategy depends, however, on the ability to design molecular assays that allow the detection of donor-specific DNA sequences. Most workers in this field have thus far used Y-chromosomal markers in situations in which the donor is male and the recipient is female *(1, 2, 4)*. This strategy would work only in the subset of transplantation cases with the required sex-mismatched donor–recipient configuration. Gadi et al. used polymorphisms in the HLA region to select PCR assays that would specifically detect the donor-derived HLA alleles *(5)*. Although this strategy has broader population coverage than the above-mentioned sex-mismatched strategy, it nonetheless requires specific assays to be designed for a particular donor–recipient pair.

In a recent publication, Snyder et al. described their massively parallel sequencing of the plasma DNA of heart transplant recipients *(6)*. They obtained a mean of $10–12 \times 10^6$ unique aligning reads per plasma sample. Concurrently, Snyder et al. also used a bead-based system for genotyping constitutional DNA from transplant donors and recipients in a genomewide manner. Using such genotyping information, these investigators then analyzed the plasma DNA–sequencing data for donor-specific alleles of single-nucleotide polymorphisms. They were then able to calculate the fractional concentration of donor-derived DNA in each plasma sample. Through ROC curve analysis, they discovered that their use of a diagnostic threshold of 1.7% for the fractional donor-derived DNA concentration enabled the detection of rejection events with a true-positive rate of 83% and a false-positive rate of 16%.

The main advantage of this method is that it can potentially be used for all genetically nonidentical donor–recipient pairs. Thus, this strategy may have a more general applicability than previous approaches based on Y-chromosomal markers *(1, 2, 4)*, and even genetic markers in the HLA region *(5)*. The potential disadvantage of the new approach may be the relative costliness of massively parallel sequencing and the relative complexity of the subsequent bioinformatics analysis compared with conventional PCR-based detection strategies. The cost issue is further compounded by the fact that testing at multiple time points may be needed for a particular transplant recipient for monitoring purposes. The costs associated with massively parallel sequencing are falling rapidly, however, and it is likely that the cost will no longer be a substantial issue in a few years' time.

Another important point is that the sequencing-based approach measures the donor-derived DNA as a fraction of the total plasma DNA *(6)*, whereas a PCR-based approach can measure an absolute concentration of donor-derived DNA in plasma *(4, 5)*. The advantage of expressing the results as a fraction is that the calculation internally controls for some of the variables involved in sample processing, e.g., DNA yield and so on. The disadvantage, on the other hand, is that if the pathologic processes being monitored (e.g., rejection)

[1] Li Ka Shing Institute of Health Sciences and [2] Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China.
* Address correspondence to the author at: Department of Chemical Pathology, Prince of Wales Hospital, 30-32 Ngan Shing St., Shatin, New Territories, Hong Kong SAR, China. Fax +852-26365090; e-mail loym@cuhk.edu.hk.
Received April 23, 2011; accepted April 26, 2011.
Previously published online at DOI: 10.1373/clinchem.2011.166686
[3] Human genes: *TSPY1*, testis specific protein, Y-linked 1; *SRY*, sex-determining region Y.

were to lead indirectly to an increase of the total plasma DNA as well as the donor-derived DNA, then the sensitivity of the approach for detecting such processes might be reduced, compared with approaches based solely on the detection of the absolute concentration of donor-derived DNA.

The true-positive and false-positive rates of the approach described by Snyder et al. are encouraging. Future work would be needed to see if these results could be further improved. One possibility whereby that can be achieved is to combine this new plasma DNA sequencing–based approach with other molecular markers for rejection. It would also be important to evaluate the stability of the diagnostic threshold concentration reported by Snyder et al. when it is applied to other sample cohorts. Parameters that would need to be addressed include the potential variation in the diagnostic threshold with regard to the ethnic origin of the patients, the immunologic compatibility of the donor and recipient, and the immunosuppressive regimen used. Given that the total DNA in a sample is an important parameter in determining the calculated fractional donor-derived DNA concentration, the efficiency of the sample-processing protocol in obtaining truly cell-free plasma is an important parameter to consider for future work. Any residual nucleated blood cells, which would be expected to be derived from the recipient, would artifactually reduce the fractional concentration of donor-derived DNA in plasma. Because Snyder et al. used archived plasma samples, these important preanalytical variables for plasma processing could not be addressed with their data set.

Snyder et al. have presented data only regarding the application of their approach to heart transplant recipients *(6)*; however, their method should also work for other types of transplantation. Given that previous work has suggested that different organs might release different amounts of DNA into the plasma *(2)*, one would predict that the diagnostic threshold concentration for detecting rejection would need to be established individually for different types of transplantation. This approach is expected also to be applicable to other bodily fluids, e.g., the urine of kidney transplantation patients, for which donor-derived DNA sequences have previously been reported.

Finally, apart from being a promising molecular diagnostic tool, the use of massively parallel sequencing of plasma DNA is expected to yield valuable information regarding the biology of plasma nucleic acids. For example, it would be interesting to investigate whether plasma DNA released by different organs exhibits different biophysical characteristics. An improved understanding of the biology of circulating nucleic acids would ultimately enhance our ability to harness its diagnostic potential.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

Employment or Leadership: Y.M.D. Lo, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: Y.M.D. Lo, Sequenom.
Stock Ownership: Y.M.D. Lo, Sequenom.
Honoraria: None declared.
Research Funding: Y.M.D. Lo, Sequenom.
Expert Testimony: None declared.

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