Molecular Testing of Urine: Catching DNA on the Way Out

There has been much recent interest in the potential diagnostic use of DNA in plasma and serum for cancer testing *(1)*, prenatal diagnosis *(2)*, and transplantation monitoring *(3)*. It has also been demonstrated that circulating DNA appears to be cleared very rapidly from the circulation *(4)*. The exact mechanism of clearance, however, has remained incompletely understood. In this issue of the Journal, Botezatu et al. *(5)* describe interesting results indicating that the kidney plays a role in the clearance of plasma DNA and that, excitingly, a proportion of the DNA is excreted in sizes detectable by molecular techniques such as PCR.

Botezatu et al. *(5)* began their investigation by demonstrating that mice injected with either purified bacteriophage DNA or irradiated Raji cells excreted detectable amounts of DNA derived from the injected materials. They went on to show that their observations could also be extended to humans by demonstrating the presence of male DNA in the urine of women transfused with male blood and in women pregnant with male fetuses *(5)*. These authors continued their line of investigation by showing that tumor-associated K-*ras* mutations were detectable in the urine of pancreatic and colorectal carcinoma patients. Overall, their results have opened up the possibility that urinary DNA analysis may have diverse clinical applications, ranging from cancer testing to prenatal diagnosis.

These exciting results have presented the scientific community with several tasks. First, these data would need to be reproduced by independent groups of investigators. Second, many technical issues would need to be addressed. For example, different DNA extraction methods would need to be evaluated for their efficiency in purifying DNA from urine. With regard to DNA extraction, it is surprising to note that Botezatu et al. *(5)* have used unfractionated urine, which presumably contains both cellular and noncellular materials. Theoretically, one would assume that DNA excreted through the kidney would exist in the noncellular fraction of urine. The suggestion by the authors *(5)* that DNA might be adsorbed onto particulate material in urine is interesting and would need to be studied in future research. Third, most of the data of Botezatu et al. are qualitative in nature. It would be interesting to measure the concentrations of target DNA in urine samples, as has been done for plasma DNA in pregnant women *(6)* and cancer patients *(7)*. Fourth, practical issues such as the hydration status of the patients and the optimal time during the day for sample collection would need to be further evaluated.

With regard to fetal DNA detection in maternal urine, Botezatu et al. *(5)* have used a highly repetitive Y-chromosomal target (*DYZ1*), with repeats up to 5000 times per male cell *(8)*. Thus, whether urinary DNA analysis is sensitive enough to detect single-copy fetal DNA sequences, such as the *RHD* gene *(2)*, remains to be demonstrated. Botezatu et al. *(5)* used nested PCR to detect fetal DNA in maternal urine, which suggests that the amount of urinary fetal DNA is low and that the extension of these results to single-copy fetal genes, with copy numbers some three orders of magnitude lower than that of *DYZ1*, may offer considerable technical challenge and may require large amounts of maternal urine. On the optimistic side, however, is the fact that the pregnant women studied by Botezatu et al. *(5)* were in the first trimester of pregnancy, a time when the concentrations of fetal DNA in maternal plasma have been shown to be relatively low compared with those at a later stage of pregnancy *(6)*. Thus, detection of DNA in the urine of women at a more advanced gestational age may be less daunting. A potential source of false positivity of prenatal diagnosis using maternal urine analysis is sexual intercourse during pregnancy, which may potentially lead to the contamination of urine samples with sperm DNA and a resulting false-positive Y-chromosomal PCR.

Botezatu et al. *(5)* have also demonstrated that their Y-chromosomal PCR system can be used to detect male DNA in the urine of women who had been transfused with male blood. It was unclear from their report what volume of blood transfusion these subjects had received. These results raise the possibility that other "iatrogenically introduced" DNA-containing materials, such as a grafted organ, may also cause the appearance of foreign DNA in the urine. This possibility may extend the previous observations of donor-derived DNA in the urine of kidney transplant recipients *(9)* to other types of transplantation, such as liver and bone marrow transplantation.

Botezatu et al. *(5)* demonstrated the potential applicability of their technique in the detection of cancer. Unlike previous work illustrating the diagnostic use of urine for cancer detection *(10, 11)*, the cancer types chosen by Botezatu et al. *(5)*, namely pancreatic and colorectal carcinomas, are not of urologic origin. Previous work has indicated that pancreatic *(12)* and colorectal *(13)* cancer cells can release tumoral DNA into the plasma. The new results by Botezatu et al. *(5)* go one step further by suggesting that tumor DNA, following its release into the blood stream, will be excreted into the urine in sizes large enough for PCR analysis. Because the current data by Botezatu et al. include only patients with relatively advanced diseases (stages III and IV), the applicability of urine DNA analysis to the detection of early nonurologic malignancies remains to be demonstrated in future studies. Alternatively, it is possible that this urinary assay may become a noninvasive indicator of the presence of advanced disease.

The results of the study by Botezatu et al. *(5)* highlight the importance of improving our understanding of the biology and metabolism of cell-free DNA in the body. The relationship between the plasma concentration of a particular DNA species and its concentration in the urine remains to be studied. Answers to this question would

require studies combining plasma and urine DNA measurements in the same cohort of patients. It would also be interesting to study the relationship between renal function/dysfunction and the excretion of DNA.

In conclusion, the report by Botezatu et al. *(5)* has shed new light on the renal excretion of DNA. Biologically, it suggests that the phenomenon of "urinary DNA chimerism" *(9)* might be more common than previously realized. For molecular diagnosis, these results suggest the exciting possibility that the noninvasive diagnosis and monitoring of many diseases may be achieved by analyzing a body fluid type that can be obtained safely and in virtually unlimited amounts.

References

1. Chen XQ, Stroun M, Magnenat J-L, Nicod LP, Kurt A-M, Lyautey J, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996;2:1033–5.
2. Lo YMD, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med 1998;339:1734–8.
3. Lo YMD, Tein MSC, Pang CCP, Yeung CK, Tong KL, Hjelm NM. Presence of donor-specific DNA in plasma of kidney and liver transplant recipients [Letter]. Lancet 1998;351:1329–30.
4. Lo YMD, Zhang J, Leung TN, Lau TK, Chang AMZ, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet 1999;64:218–24.
5. Botezatu I, Serdyuk O, Potapova G, Shelepov V, Alechina R, Molyaka Y, et al. Genetic analysis of DNA excreted in urine: a new approach for detecting specific genomic DNA sequences from dying cells in an organism. Clin Chem 2000;46:1078–84.
6. Lo YMD, Tein MSC, Lau TK, Haines CJ, Leung TN, Poon PMK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768–75.
7. Lo YMD, Chan LYS, Lo KW, Leung SF, Zhang J, Chan ATC, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 1999;59:1188–91.
8. Nakahori Y, Mitani K, Yamada M, Nakagome Y. A human Y chromosome specific DNA family (DYZ1) consists of a tandem array of pentanucleotides. Nucleic Acids Res 1986;14:7569–80.
9. Zhang J, Tong KL, Li PKT, Chan AYW, Yeung CK, Pang CCP, et al. Presence of donor- and recipient-derived DNA in cell-free urine samples of renal transplantation recipients: urinary DNA chimerism. Clin Chem 1999;45:1741–6.
10. Mao L. Genetic alterations as clonal markers for bladder cancer detection in urine. J Cell Biochem Suppl 1996;25:191–6.
11. Eisenberger CF, Schoenberg M, Enger C, Hortopan S, Shah S, Chow NH, et al. Diagnosis of renal cancer by molecular urinalysis. J Natl Cancer Inst 1999;91:2028–32.
12. Mulcahy HE, Lyautey J, Lederrey C, Chen XQ, Anker P, Alstead EM, et al. A prospective study of K-*ras* mutations in the plasma of pancreatic cancer patients. Clin Cancer Res 1998;4:271–5.
13. Anker P, Lefort F, Vasioukhin V, Lyautey J, Lederrey C, Chen XQ, et al. K-*ras* mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology 1997;112:1114–20.

Y.M. Dennis Lo

Department of Chemical Pathology
The Chinese University of Hong Kong
Prince of Wales Hospital
Shatin, New Territories
Hong Kong Special Administrative Region
Fax 852-2194-6171
E-mail loym@cuhk.edu.hk