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Transplant assessment

Now quantify the risk

A cfDNA background check everytime

May 2020

How quantifying background cell-free DNA (cfDNA) is raising the bar for precision in rejection assessment

Introduction

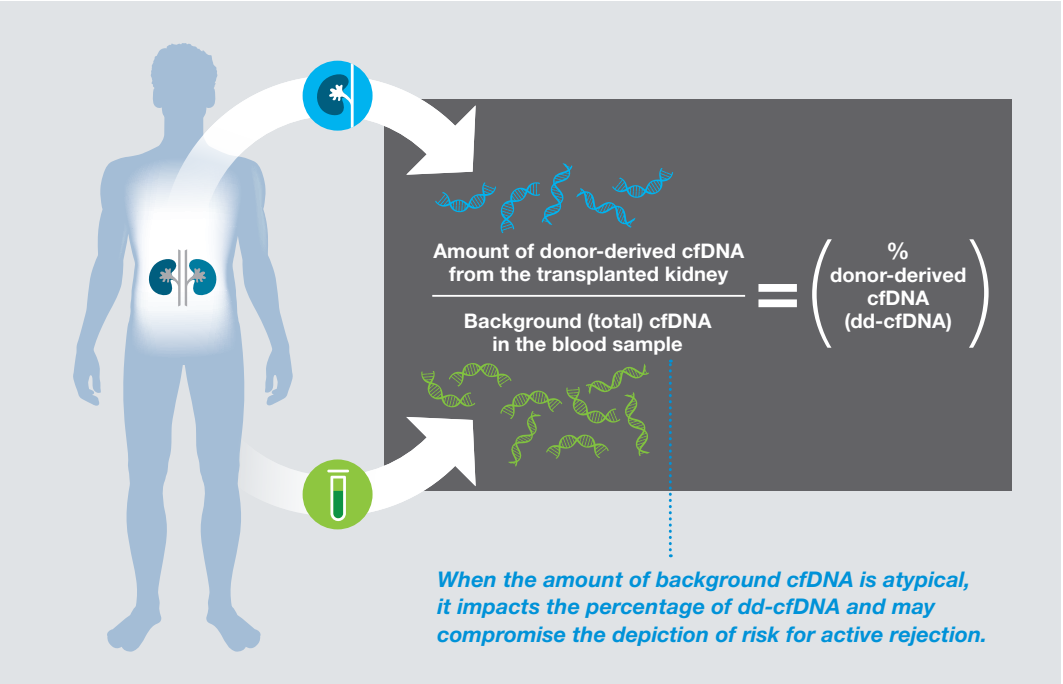
Non-invasive monitoring using cell-free DNA (cfDNA) technology relies on established methods to distinguish self from non-self DNA that is in the blood stream such as DNA from a fetus (prenatal), tumor (oncology), or organ donor (transplantation).[1–16] Donor-derived cfDNA (dd-cfDNA) is a proven biomarker in kidney and heart transplantation for identifying active rejection.[1–6,13–16] Existing commercial assays report dd-cfDNA results as a percentage of total cfDNA. However, results reported in this manner may not provide a full picture of rejection risk due to background cfDNA levels that can be affected by many factors. Atypically high levels of recipient cfDNA may lead to a decreased dd-cfDNA proportion, and a potential false negative interpretation; less frequently, atypically low cfDNA levels can lead to false positive results.

Natera's Prospera™ transplant assessment test is powered by highly optimized, proprietary cfDNA technology. It has now been enhanced with an exclusive technique, making Prospera the first test of its kind to quantify absolute background cfDNA.

This enhancement benefits physicians by identifying patients with atypical background cfDNA levels – thereby flagging them as at-risk for false-negative reporting and potentially missed rejections.

More? Less? Why the amount of background cfDNA matters

Background cfDNA originates from the transplant recipient and is naturally occurring in variable amounts within the plasma.



About the Prospera transplant assessment test

Prospera assesses all types of kidney transplant rejection[1] with great precision.[2,3] From a single blood draw, Prospera measures the amount of donor cfDNA from the transplanted kidney in the patient's blood. Using more than 13,000 single-nucleotide polymorphisms (SNPs) and advanced bioinformatics, the assay can differentiate donor and recipient cfDNA to provide a result as a percentage of dd-cfDNA in a transplant recipient's blood. A greater percentage of dd-cfDNA in a recipient's blood may signify that the transplanted organ is shedding more DNA than ideal, thereby indicating an increased risk of rejection.

Prospera's performance was evaluated in a blinded, large scale study of 217 biopsy-matched renal allograft plasma samples using a prospectively determined cut-off of 1% dd-cfDNA or greater.[1] Sigdel et al[1] demonstrated Prospera's superior accuracy in identifying active rejection over current standard-of-care biomarkers (estimated glomerular filtration rate and serum creatinine). Comparative statistics for Prospera include sensitivity of 89% vs 52%; specificity of 73% vs 68% and area under the curve (AUC) of 0.87 vs 0.68, respectively.[1] Test performance in the validation study was independent of donor type (related/unrelated, living/deceased), rejection type (antibody mediated rejection/T cell-mediated rejection/combination) and clinical presentation (clinical/subclinical).[1]

The first to optimize for precision and accuracy

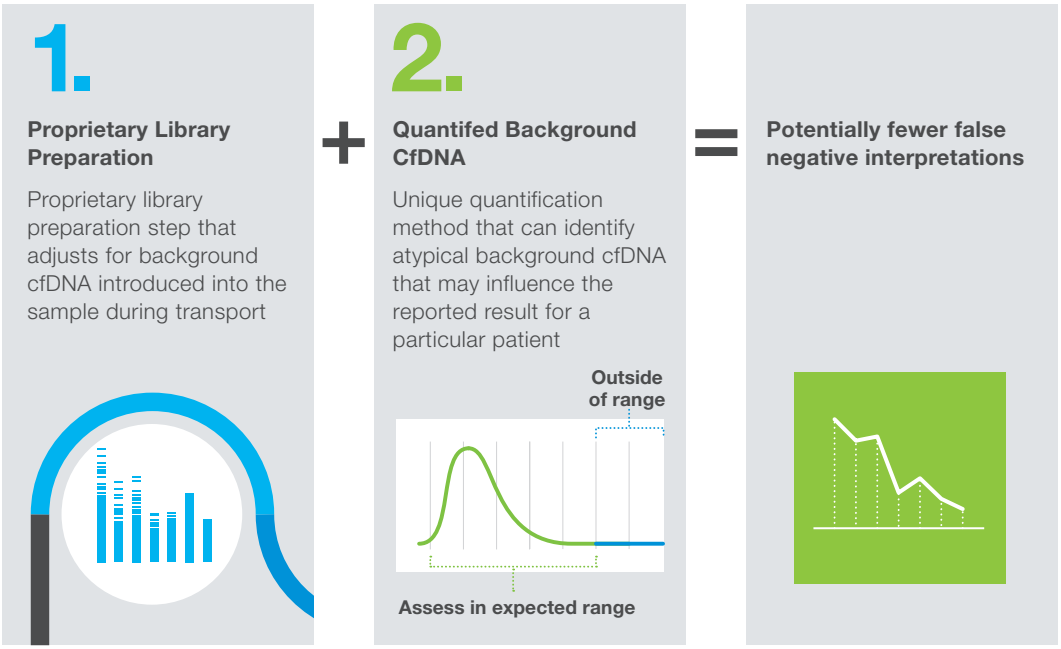
Natera has processed more than two million cfDNA tests and is the first laboratory to introduce two novel techniques that together deliver increased precision and further expand confidence in Prospera results.

Technique 1: Proprietary library preparation. This technique results in higher yield, higher quality DNA than standard cfDNA tests. It accounts for additional cfDNA that may be released into the sample during collection and transport.

Technique 2: Quantification of background cfDNA. This technique identifies atypical levels of background cfDNA that may influence the reported result for a particular patient.

Applying both techniques may flag fewer false negative interpretations.

Figure 1: Two new techniques for enhanced Prospera results

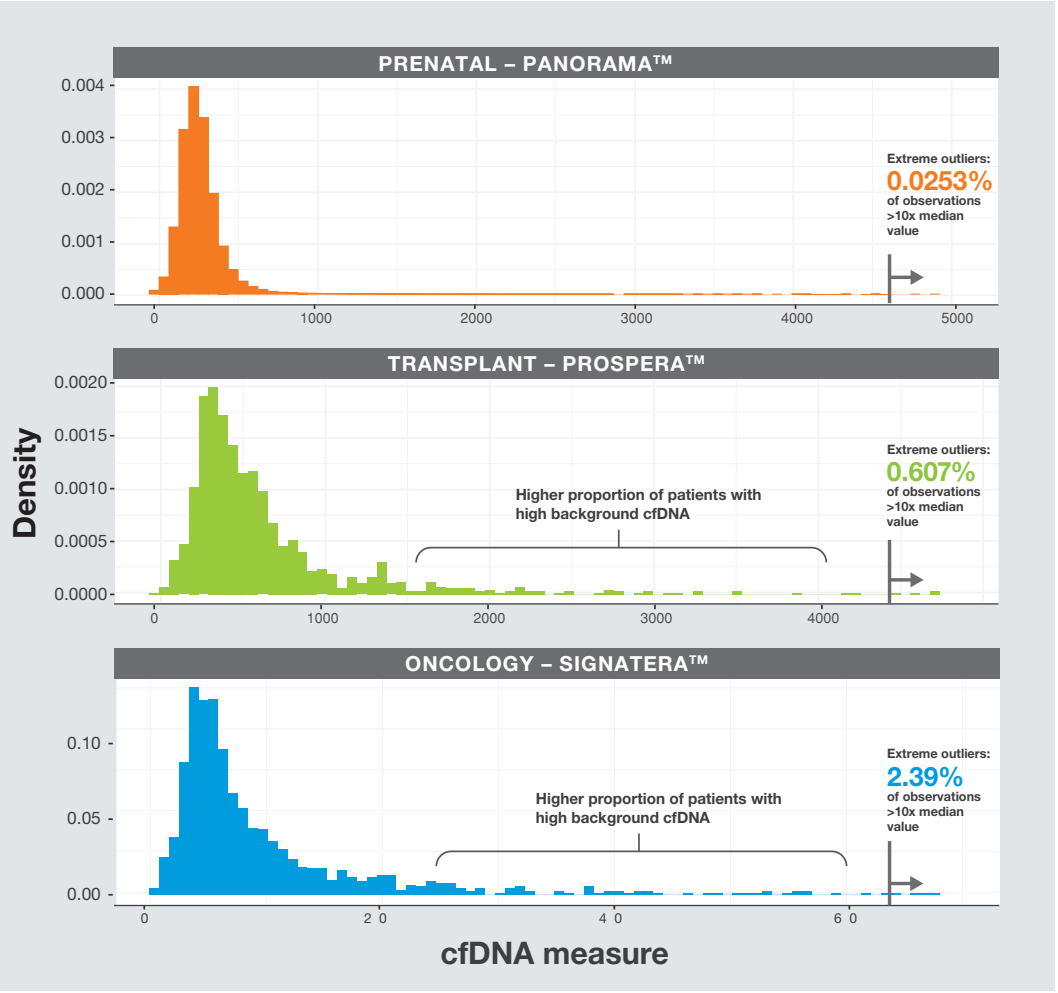


Enabling the quantification of background cfDNA

Natera scientists reviewed more than 20,000 cfDNA cases in prenatal, ~ 1,000 cfDNA cases in oncology, and ~1,000 cfDNA cases in transplantation to identify an "expected range" of background DNA in a patient.

The graphs shown in Figure 2 compare results from three sets of tests.

Figure 2: Defining "expected range" of background cfDNA

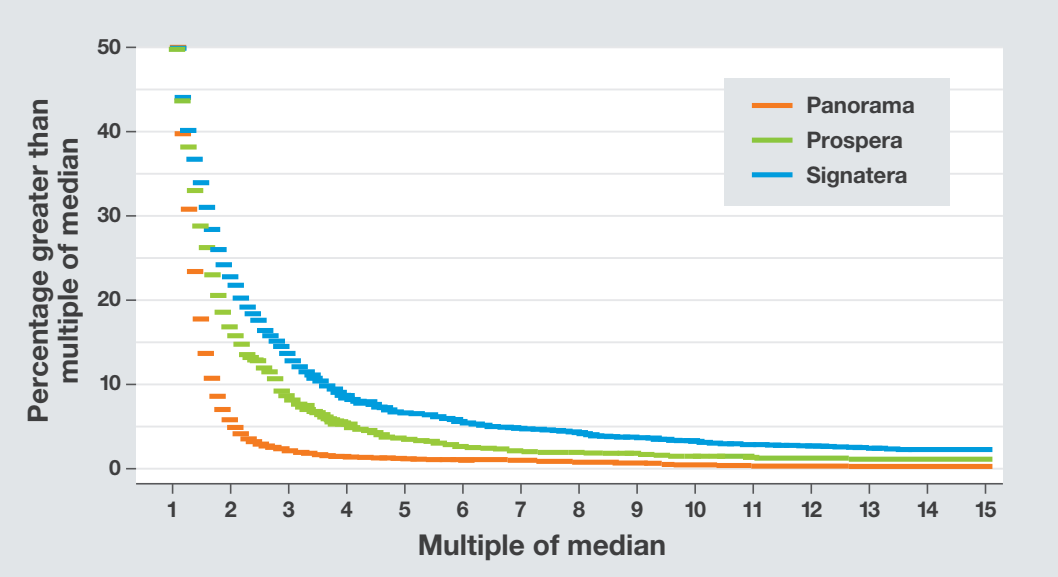


The results from Panorama™, a non-invasive prenatal screening test to identify chromosomal variants, represent an overall healthy population of pregnant women. These data sets were compared to results from cancer patients using the Signatera™ minimal residual disease test and an indirect measurement of total background cfDNA results from Prospera on transplant patients.

Further analysis of the three data sets showed that the transplant and oncology patients were more likely to have very high cfDNA measurements relative to the median. For example, the fraction of transplant patients with cfDNA measurement more than 8 times the median was about 33 times greater compared to the fraction of Panorama patients. Figure 3 shows the percentage of patients above a certain multiple of the median, for the three patient groups. The actual medians are approximately: 487.98 per mL for Prospera ratio, 266.43 per mL for Panorama ratio, and 6.825 ng/mL for Signatera total cfDNA.

Figure 3:
Direct and indirect measurements of background cfDNA

How heavy are the tails for various tail lengths

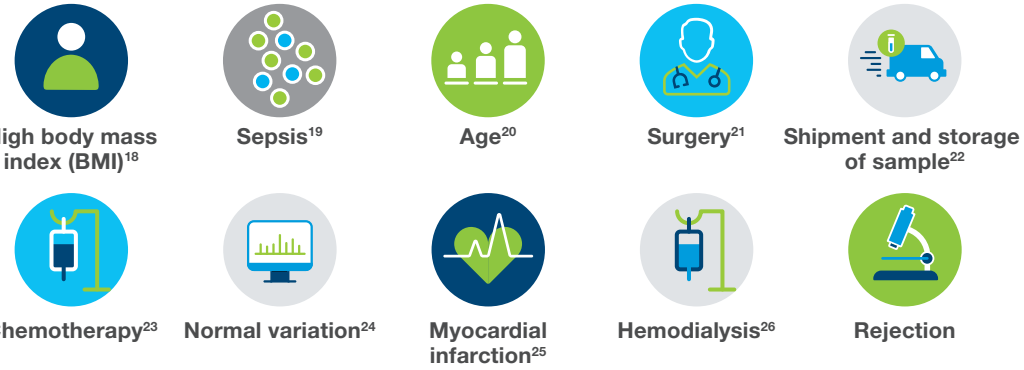


These data sets suggest that patients with certain medical problems such as cancer and kidney disease may have unusually high background cfDNA levels. Conversely, a healthy population of pregnant women was used to define the "normal" range. **This finding painted a picture we could not ignore: A fraction-based rejection assay may not be sufficiently precise for all transplant patients.**

Potential factors influencing background DNA

A recent literature review highlighted several factors that may cause atypical levels of background cfDNA in a patient's sample. In a review of 80 patients with severe sepsis, total cfDNA levels had a better prognostic utility than MODS or APACHE II scores, with an AUC for ICU mortality of 0.97.[27] This suggests that infection can elevate cfDNA levels. Further, a study in mice showed that fat cells can degenerate and elevate cfDNA levels, which in turn can directly cause inflammation.[28] Also, cfDNA levels seem to increase after dialysis and may predict mortality in these patients.[28,29]

Preliminary data suggest that any inflammatory process in the body including infection can cause a surge in background cfDNA.



Case studies support findings

Natera's review of internal data revealed similar drivers contributing to the variability of cfDNA levels, including viral infection.

Case Study 1:

Meet Kirk*

The patient: A male in his late 70s with end-stage renal disease (ESRD)

The journey:

- In mid-2018, the patient underwent a kidney transplant.
- At six months post-transplant surgery, his creatinine level was elevated, indicating acute T cell-mediated rejection (TCMR).
- At seven months post-transplant surgery, he tested positive for BK viremia, which was immediately treated and resolved.
- At 14 months post transplant surgery, he was admitted for herpetic and cytomegalovirus (CMV) esophagitis and was treated with intravenous ganciclovir.

Clinical assessment with Prospera:

- The Prospera result revealed a low donor-derived cell-free DNA (dd-cfDNA) fraction at 0.38%, indicating a decreased risk for active rejection.
- Further Prospera analysis quantified background cfDNA, revealing a level 21x the median – and thereby flagging an increased risk of a false-negative interpretation.
- Based on Prospera's enhanced reporting, percutaneous kidney transplant biopsy was performed; the result confirmed chronic cellular rejection (via Banff criteria).

The takeaway:

Viral infections can cause an atypical increase in recipient background cfDNA. This inflation may lead to an artificially deflated percentage of donor-derived cfDNA.

Prospera's novel ability to quantify background cfDNA highlighted an increased risk for a false-negative interpretation. This prompted a crucial biopsy confirming active rejection that may have otherwise been missed.

Case Study 2:

Meet Janice*

The patient: A female in her early 60s with end-stage renal disease

The journey:

- In early 2017, she received a kidney transplant from a deceased donor.
- Three years post-transplant surgery, she was assessed with Prospera during a routine visit.

Clinical assessment with Prospera:

- Prospera result showed a donor fraction of 0.28%, potentially a decreased risk for active rejection.
- The report also flagged atypical background cfDNA levels that were elevated at ~ 7x the median.
- The resulting percutaneous kidney transplant biopsy revealed BK virus-associated nephropathy and T cell-mediated rejection.

The takeaway:

BK virus-associated active injury may contribute to atypical background cfDNA levels.

Prospera's latest enhancement allows for physicians to more effectively identify active rejection that would have otherwise been missed.

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Case Study 3:

Meet Scotty*

The patient: A male in his early 50s with end-stage renal disease

The journey:

- In late 2019, he obtained a kidney transplant from an unrelated, living donor.
- One month post-transplant, he was diagnosed with dengue fever, followed by acute allograft dysfunction.
- At 6 months post-transplant, a biopsy was performed revealing active antibody-mediated rejection. He was then treated with plasmapheresis and intravenous immunoglobulin with clinical resolution.

Clinical assessment with Prospera:

- At 7 months post-transplant, he received a Prospera result of 0.16% dd-cfDNA level, indicative of a decreased risk for active rejection.
- The Prospera result also revealed a heightened level of background cfDNA at ~13X the median.
- A biopsy thereafter showed resolution of ABMR and borderline acute cellular rejection.

The takeaway:

For the first time, further evaluation of background cfDNA levels enabled the physician to identify signs of borderline acute cellular rejection.

This additional information by Prospera can provide a more complete clinical assessment of your transplant patient.

Case Study 4:

Meet Leia*

The patient: A female in her late 50s with end-stage renal disease secondary to polycystic kidney disease (PKD)

The journey:

- In late 2018, she received a kidney transplant from a deceased donor.
- At 11 months post-transplant, she presented with four days of worsening diffuse muscle pain.
- With normal labs in the prior week, her symptoms progressed with a temperature of 101°F so she visited her local physician and was sent to the local emergency room.
- After being tested as positive by COVID-19 nasopharyngeal swab, she was soon transferred to her transplant center where her respiratory status worsened and she was intubated.
- Progressing to septic shock requiring vasopressor therapy, her renal function deteriorated and immunosuppression dosages were closely managed.

Clinical assessment with Prospera:

- Prospera was used to assess rejection status on the 20th day of her hospital stay
- The Prospera result showed 0.07% dd-cfDNA with a heightened level of background cfDNA at ~57x the median.
- A second Prospera test was drawn on the 25th day of her hospital stay with a result of 0.25% dd-cfDNA and a decreased level of background cfDNA at ~ 15x the median.

The takeaway:

COVID-19 may cause very elevated background cfDNA. Therefore, these patients are at-risk for a false negative interpretation, especially when immunosuppression is reduced in response to the infection.

By reporting high background levels, Natera proactively alerts the physician if the result may yield a false negative in a high-risk patient.

*** Deidentified patient names and details**

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References

1 Sigdel TK, Archila FA, Constantin T, et al. Optimizing detection of kidney transplant injury by assessment of donor-derived cell-free DNA via massively multiplex PCR. J Clin Med. 2019;8(1):19.
2 Altug Y, Liang N, Ram R, et al. Analytical validation of a single-nucleotide polymorphism-based donor-derived cell-free DNA assay for detecting rejection in kidney transplant patients. Transplantation, 2019
3 Grskovic M, Hiller DJ, Eubank LA, et al. Validation of a clinical-grade assay to measure donor-derived cell-free DNA in solid organ transplant recipients. J Mol Diagn. 2016;18(6):890-902.
4 Bromberg JS, Brennan DC, Poggio E, et al. Biological variation of donor-derived cell-free DNA in renal transplant recipients: clinical implications. J Appl Lab Med. 2017;2(3):309-321.
5 Bloom RD, Bromberg JS, Poggio ED, et al. Cell-free DNA and active rejection in kidney allografts. J Am Soc Nephrol. 2017;28(7):2221-2232. doi: 10.1681/ASN.2016091034.
6 Snyder, et al. Universal noninvasive detection of solid organ transplant rejection. Proc. Natl. Acad. Sci. USA 2011, 108, 6229 - 6234
7 Palomaki, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med. 2011 Nov;13(11):913-20
8 Sparks, et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012, 206, P319.E1-319.E9
9 Newman, et al. An ultrasensitive method for quantitating circulating tumor DNA with... Nat Med 2014, 2014 May;20(5):548-54
10 Pergament, et al. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. Obstet. Gynecol. 2014, 124 Pt 1, 210 - 218
11 Abbosh, et al. Phylogenetic ctDNA analysis depict early-stage lung cancer evolution. Nature 2017, 545, 446 - 451
12 Lo, et al. Biomarkers for kidney transplant rejection. Nat. Rev. Nephrol. 2014, 10, 215 - 225
13 Beck, et al. Digital droplet PCR for rapid quantification of donor DNA in the circulation of transplant recipients as a potential universal biomarker of graft injury. Clin. Chem. 2013, 59, 1732-1741
14 De Vlaminck, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci. Transl. Med. 2014, 6, 241ra77
15 Hidestrand, et al. Highly sensitive noninvasive cardiac transplant rejection monitoring using targeted quantification of donor-specific cell-free deoxyribonucleic acid. J Am Coll Cardiol. 2014;63(12):1224–1226. doi:10.1016/j.jacc.2013.09.029
16 Huang, et al. Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. Transplantation. 2019, doi: 10.1111/ajt.15289.
17 Philippe Gauthier, et al. Factors influencing background cfDNA levels: implications for dd-cfDNA assessment in transplant patients. ATC Poster Accepted, May 2020
18 Vora NL, Johnson KL, Basu S, Catalano PM, Hauguel-De Mouzon S, Bianchi DW. A multifactorial relationship exists between total circulating cell-free DNA levels and maternal BMI. Prenat Diagn. 2012;32(9):912–914. doi:10.1002/pd.3919
19 Ahmed AI, Soliman RA, Samir S Cell Free DNA and Procalcitonin as Early Markers of Complications in ICU Patients with Multiple Trauma and Major Surgery. Clin Lab. 2016 Dec 1;62(12):2395-2404
20 Anker, P., Stroun, M., 2000. Circulating DNA in plasma or serum. Medicina (B Aires) 60, 99–702
21 Yu Qi,1 Tokujiro Uchida,1 Mamoru Yamamoto,1 Yudai Yamamoto,1 Koji Kido,1 Hiroyuki Ito,1 Nagara Ohno,2 Miho Asahara,2 Yoshitsugu Yamada,2 Osamu Yamaguchi,3 Chieko Mitaka,1 Makoto Tomita,4 and Koshi Makita Perioperative Elevation in Cell-Free DNA Levels in Patients Undergoing Cardiac Surgery: Possible Contribution of Neutrophil Extracellular Traps to Perioperative Renal Dysfunction. Anesthesiology Research and Practice Volume 2016, Article ID 2794364, 11 pages
22 Page, K., Guttery, D.S., Zahra, N., Primrose, L., Elshaw, S.R., Pringle, J.H., Blighe, K., Marchese, S.D., Hills, A., Woodley, L., Stebbing, J., Coombes, R.C., Shaw, J.A., 2013. Influence of plasma processing on recovery and analysis of circulating nucleic acids. PLoS One 8, e77963.
23 SWYSTUN, MUKHERJEE , LIAW. Breast cancer chemotherapy induces the release of cell-free DNA, a novel procoagulant stimulus. Journal of Thrombosis and Haemostasis,9: 2313–2321
24 Stroun, M., Lyautey, J., Lederrey, C., Olson-Sand, A., Anker, P., 2001b. About the possible origin and mechanism of circulating DNA. apoptosis and active DNA release. Clin Chim Acta 313, 139–142.
25 Chang, Rhu-Hsin, Tsu-Lan et al. Elevated cell-free serum DNA detected in patients with myocardial infarction. Chimica Acta Volume 327, Issues 1–2, January 2003, Pages 95-101
26 Tovbin, Novack, Wiessman, Elkadir, Zlotnik, Douvdevani. Circulating cell-free DNA in hemodialysis patients predicts mortality Nephrology Dialysis Transplantation, Volume 27, Issue 10, October 2012, Pages 3929–3935
27 Dwivedi DJ, Toltl LJ, Swystun LL, et al. Prognostic utility and characterization of cell-free DNA in patients with severe sepsis. Crit Care 2012;16:R151
28 Nishimoto S, Fukuda D, Higashikuni Y, et al. Obesity-induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance. Sci Adv 2016;2:e1501332

Conclusion

Given the variability of cfDNA in several pathogenic states and its role as an inflammatory mediator, a dd-cfDNA-based assay that expresses results as a percentage of the total "background" cfDNA must necessarily be inaccurate in some patients. This is a metric that is variable and may be influenced by clinical or treatment-related factors. Based on published studies and data derived from the large number of tests performed by Natera, transplant patients appear to have more variability in background cfDNA levels than a non-transplant population. Variability in this metric may be influenced by clinical or treatment-related factors.

More specifically, recent data from Natera suggests a correlation between background cfDNA levels and multiple factors, including patient weight, medications, recent surgery and medical complications. For example, patients with viral infections may have atypically high background cfDNA levels.

If using a dd-cfDNA-based assay that expresses results as a percentage of total background cfDNA, it is important to flag patients with atypical levels of background cfDNA, as this will affect the final dd-cfDNA result. Most commonly, an unusually high level can result in an artificially low dd-cfDNA result, increasing the risk for false-negative interpretations and missed opportunities to preemptively spot rejections.

As such, responsible laboratories leveraging dd-cfDNA technology should consider both the proportion of dd-cfDNA and the background cfDNA levels when reporting results.

Learn more about Natera's latest enhancement:

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