AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

Pearl Title: Cell Free DNA: General Principles and Clinical Applications

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CCTC Clinical Chemistry Trainee Council

Cell Free DNA

First identified in peripheral blood in 1948

Source: cellular apoptosis/ necrosis

- Cell free DNA (cfDNA) released by dying cells
- Free DNA is rapidly degraded; protein bound is preserved
- Size distribution of cfDNA- single or multiple nucleoprotein complexes
- Typically 160-200 bp in length
- Kinetics/variables- unknown

Terminologies

cfDNA

- Any free DNA in serum

cffDNA

- Cell free fetal DNA

ctDNA

- Circulating tumor DNA

Specimen Collection

Fixative-containing blood collection tubes

- Up to 7 days at room temperature
- Expensive

EDTA

- Processing preferably within 6 hour
- Stable up to 48 hours
- Lysis of normal cells- release of normal cfDNA dilutes out cfDNA of interest

Cf DNA: Clinical Applications

Prenatal

- Fetal RhD genotyping
- Fetal sex determination for sex linked disorders
- Chromosome aneuploidy detection
- Monogenic disorders

Oncology

- Cancer detection/ monitoring

Others

- Transplantation
- Autoimmune diseases

Non-Invasive Prenatal Screening

cffDNA

- Result of apoptosis of placental cells
- Highly fragmented
- 6-20% of circulating DNA in maternal blood
- Remaining cfDNA- maternal

Biological factors

- Maternal BMI
- Gestational age
- Placental health

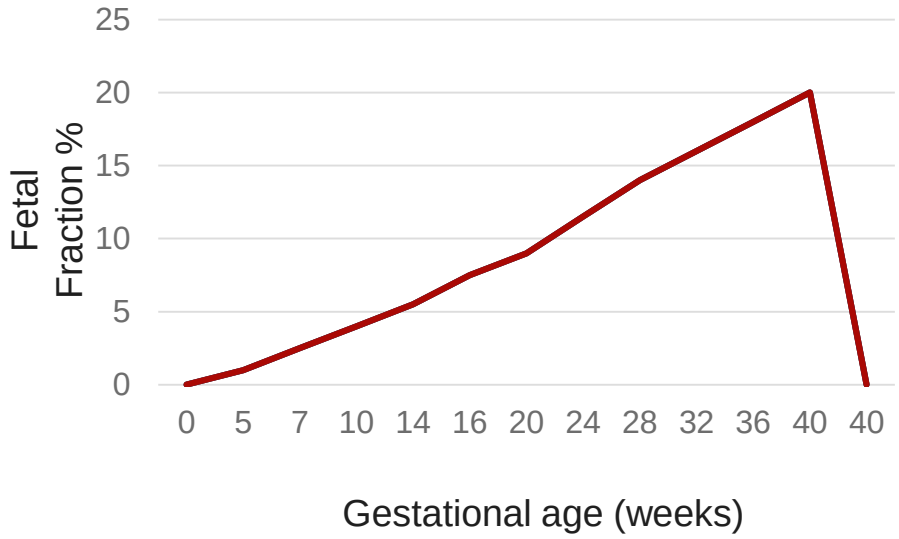
Fetal fraction of 4%- regarded to be minimal fraction needed for reliable results

cffDNA Time Course

Detectable 5 weeks after conception

Disappears within a few hours after delivery

Most laboratories offer testing at 9-10 weeks or later



cffDNA Analysis

SNP-based

- Accuracy dependent on number of SNPs analyzed
- Distinguishes between maternal and fetal sources
- Does not detect off-target abnormalities

Whole genome: shotgun sequencing

- Compares number of chromosome of interest to number expected
- Dosage information on any chromosome

Quantification of cffDNA

Sequencing data

- Reads from X or Y chromosome/ aneuploid chromosome
- Reads in benign or pathogenic CNVs
- Single nucleotide polymorphisms (SNPs)

Prenatal Screening: Constitutional disorders

Aneuploidy
- Trisomy 21
- Trisomy 13
- Trisomy 18
- Sex chromosome aneuplodies

Microdeletions/ duplications
- Cri du Chat Syndrome (5p deletion)
- DiGeorge Syndrome (22q11.2 deletion)
- Prader Willi/Angelman- 15q
- 1p36 deletion
- 4p deletion
- Cytogenetically visible imbalances (translocations)

Triploidy

NIPS: Sensitivity and Specificity

Common Aneuploidies	Sex Chromosome Aneuploidies
T21 > 99%	Male > 99%
T13 > 80%	Female > 97%
T18 > 97%	

Average false-positive rate for common aneuplodies < 1-2 per 1000 (specificity 99.8- 99.9%)

Positive and negative predictive values are affected by disease prevalence

Microdeletions/ duplications

- Individually rare
- Individual risk of having a fetus affected with a microdeletion is more than classic trisomies, especially in women < 35
- Larger deletions are more effectively detected

False Positives

- Confined placental mosaicism
- Vanishing twin
- Maternal neoplasm
- Maternally derived genetic aberrations
- Maternal mosaicism

Discordant results between NIPS and true fetal genotype are unavoidable

NIPS: Key Concepts

- Disorders screened for are rare
- Positive result does not necessarily mean an affected pregnancy
- With NIPS as a secondary test for positive combined or sequential screens
  - > 2% residual risk of having a fetus with a chromosome abnormity following a negative cell free test
- Women ≥ 35: NIPS will detect only ~ 80% of chromosomal aneuploidies

cfDNA in Oncology

Diagnostic

- Tumor specific changes/ mutations
- Early detection
- Minimal residual disease- if disease free after curative surgery
- RNA and DNA methylation patterns- tissue specific (epigenetic signatures)

Prognostic

- Risk of recurrence
- Tumor burden changes

Predictive

- Clinically actionable variants
- Treatment response
- Emerging resistant mutations
- Molecular heterogeneity
- Tumor dynamics

Tissue Diagnosis and ctDNA

Tissue diagnosis

- Tumoral heterogeneity- between tissues, within a given tissue type
- Need for invasive procedures
- Contamination with normal cells

ctDNA/ Liquid Biopsies

- Exquisite specificity
- Captures tumor heterogeneity
- Sensitivity for early stage tumor detection
- Rapid turnaround

ctDNA Detection

Targeted

- ddPCR, NGS, SNP arrays, BEAMing

Genome-wide

- Tumor specific alterations
- De novo discovery of genetic changes underlying therapy resistance
- New actionable targets

Fraction of ctDNA- correlates with cell turnover, varies between tumors, 0.01-90%

FDA approved markers

Lung carcinoma

- EGFR Mutation Test v2 for exon 19 deletion and L858R (at diagnosis); T790M (at relapse)

Colorectal cancer screening

- Methylation based markers
  - *SEPT9*
  - *NDRG4*
  - *BMP3*

Transplant Monitoring

Donor derived DNA in plasma of kidney and liver transplants- 1998

cfDNA in transplants

- L- shaped curve- high percentage in immediate post-engraftment phase, followed by rapid decrease to baseline level
- Kinetics of cfDNA is different across different organ transplants
- Normal dynamics for each type of transplant need to be established
- Quantification of donor specific DNA
  - HLA DNA and copy number polymorphisms
  - SNP distribution in donor and recipient

Applications

- Guide therapeutic decisions: antirejection drug dosing, response to therapy
- Opportunistic infections
- Donor derived mitochondrial cfDNA- more abundant, damage associated pattern
- Epigenetic markers of cfDNA- cellular source of DNA

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Disclosures/Potential Conflicts of Interest

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