Volume 1
Issue 1
September 2023

UNI DIGEST

Unveiling the power of pathology

Every specimen tells a story, and it is our job to decipher the narrative and find the answers that can make a difference.

EDITOR'S COLUMN

Dear All,

We are thrilled to present the inaugural edition of our newsletter **UNI DIGEST**. As a country's leading institution that combines exceptional skills with advanced technology, we are committed to providing the highest quality diagnostic services and comprehensive oncology care. With state-of-the-art technology at our disposal, we can harness the power of precision medicine to tailor treatment strategies to individual patients, leading to improved outcomes and enhanced quality of life. Our team of dedicated experts, armed with their profound knowledge and expertise, strives to unravel the mysteries of disease and pave the way for innovative treatments.

We believe that pathology serves as a powerful tool in understanding the intricacies of diseases. It allows us to delve deep into the molecular and cellular levels, deciphering the underlying mechanisms that drive illness. In this newsletter, we aim to keep you informed about the groundbreaking work happening within our center, and the latest advancements in pathology, microbiology, molecular, genomics, and much more. Our team of dedicated experts, armed with their profound knowledge and expertise, strives to unravel the mysteries of disease and pave the way for innovative treatments.

We aim to empower healthcare professionals with the knowledge, bridging the gap between medical expertise and understanding. As we embark on this exciting journey, we invite you to be an active part of our community, as together we can shape the future of diagnostics.

Warm regards,
Dr. Jwalant Shah, MD & CEO
Unipath Specialty Laboratory, Ahmedabad

EDITOR'S NOTE

Dear All,

It's my pleasure to extend a warm Welcome to each one of you for the **UNI DIGEST** newsletter, dedicated to exploring two crucial areas in diagnostics - Infections and cancer genomics. Through the latest research, breakthroughs, and innovative approaches we aim to shed light on how these fields are transforming the diagnosis & and management of infectious diseases, as well as unraveling the intricacies of cancer at the genomic level. By exploring these areas, we hope to foster a deeper understanding and facilitate the development of targeted therapies, ultimately improving patient outcomes.

I am sure our esteemed authors and valuable contributions will serve as an invaluable resource for healthcare professionals, researchers, and students seeking to deepen their understanding of these intricate topics. Our content is meticulously curated to provide you with the latest updates and foster a multidisciplinary approach, empowering you to make informed decisions and deliver the highest standard of care.

Your engagement and feedback is of utmost importance to us. We are eager to hear your thoughts and suggestions. Together, we can foster a collaborative environment that propels diagnostics forward, enhancing patient care.

Once again, I extend my heartfelt welcome to each one of you. Let us embark on this journey together, while striving for excellence in diagnostics.

Warm regards,
Dr. Ravi Gaur, MD
Chairman Medical Advisory Committee
Unipath Specialty Laboratory, Ahmedabad

INSIDE STORIES

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Development and Validation of Kinase domain detection Assay by NGS method

Dr. Spandan Chaudhary, Department of Molecular Genetics,
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Management and long-term survival of CML patients have been significantly changed since the emergence and use of three generations of tyrosine kinase inhibitors (TKIs). TKIs target the abnormal BCR: ABL1 protein that causes uncontrolled CML cell growth and block its function, causing the CML cells to die. The first therapy given for a disease is called “initial” or “first-line” treatment. The following four TKI drugs are approved as first-line treatment for chronic phase CML: Imatinib, Dasatinib, Nilotinib, and Bosutinib. In approximately, 33% of patients who experience resistance to first-line therapy, and in up to 50% of patients who experience resistance to second or subsequent-line therapy, point mutations in the ABL1 kinase domain (KD) that impair TKI binding can be detected.

Mutations may arise at critical contact points between the inhibitor and its target or in key regions of the KD, namely the phosphate-binding loop (P-loop), the catalytic cleft, or the activation loop (A-loop). Various mechanisms lead to a decrease or loss of response to TKIs, but the acquisition of point mutations in the BCR-ABL1 kinase domain (KD) is the most important and probably the only actionable one. Mutations make the drug ineffective in obtaining a deep clearance of cells with BCR-ABL1 fusion which slows down the clinical response and also accelerates the acquisition of additional mutations. Resulting effect can be a clonal complexity, in some patients, which is a difficult phenomenon to address therapeutically.

This is the reason that the European Leukaemia Net (ELN) and the National Comprehensive Cancer Network both have recommended screening for mutations in case of failure and warning of response to the drug under the treatment. Several assays have been designed and validated for KD-resistant mutation detection in patients with CML but SS is the current gold standard method.

SS is a faster and more cost-effective method, but, due to low sensitivity with the mutation detection limit of 10% to 20%. Sanger method provides only rough estimates of mutated clone abundance, and it is also a fact that it cannot differentiate between polyclonal and compound mutations. As an alternative, next-generation sequencing (NGS) technology has proven advantages like depth and massive parallel approach. Sequencing of multiple fragments together at a significant depth makes the NGS a very suitable method to detect even multiple mutations at greater sensitivity. Studies have already established that NGS can detect resistant mutation 12-15 months earlier than Sanger.

There is no commercial kit or assay with either European Conformity (CE)-marked for in vitro diagnosis (CE-IVD) or Food and Drug Administration (FDA)-approved commercial assay available for NGS-based BCR-ABL1 KD mutation. Available myeloid panels from vendors like Thermofisher, Qiagen, Illumina, Archer, etc. do not enrich the transcript P210 or P190 before sequencing which ultimately dilutes the mutations down to a level that might be undetectable even by NGS.

The protocol presented in this study is very fast, accurate, reproducible, and easy to implement for any lab that routinely uses any of the assays on the Ion torrent platform. The derived sensitivity of the assay is 2% We have developed an in-house assay to detect kinase domain mutation at the sensitivity of 2% by the NGS method. This method uses RNA as the starting material followed by nested PCR to amplify fusion transcript which is subsequently used as a template for NGS.

Using this assay, we have already seen an advantage over Sanger and already have delivered results for more than 130 clinical samples to date.

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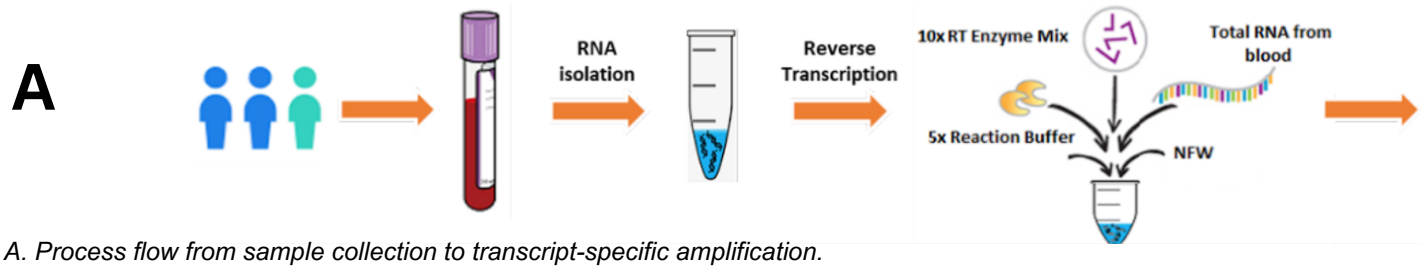
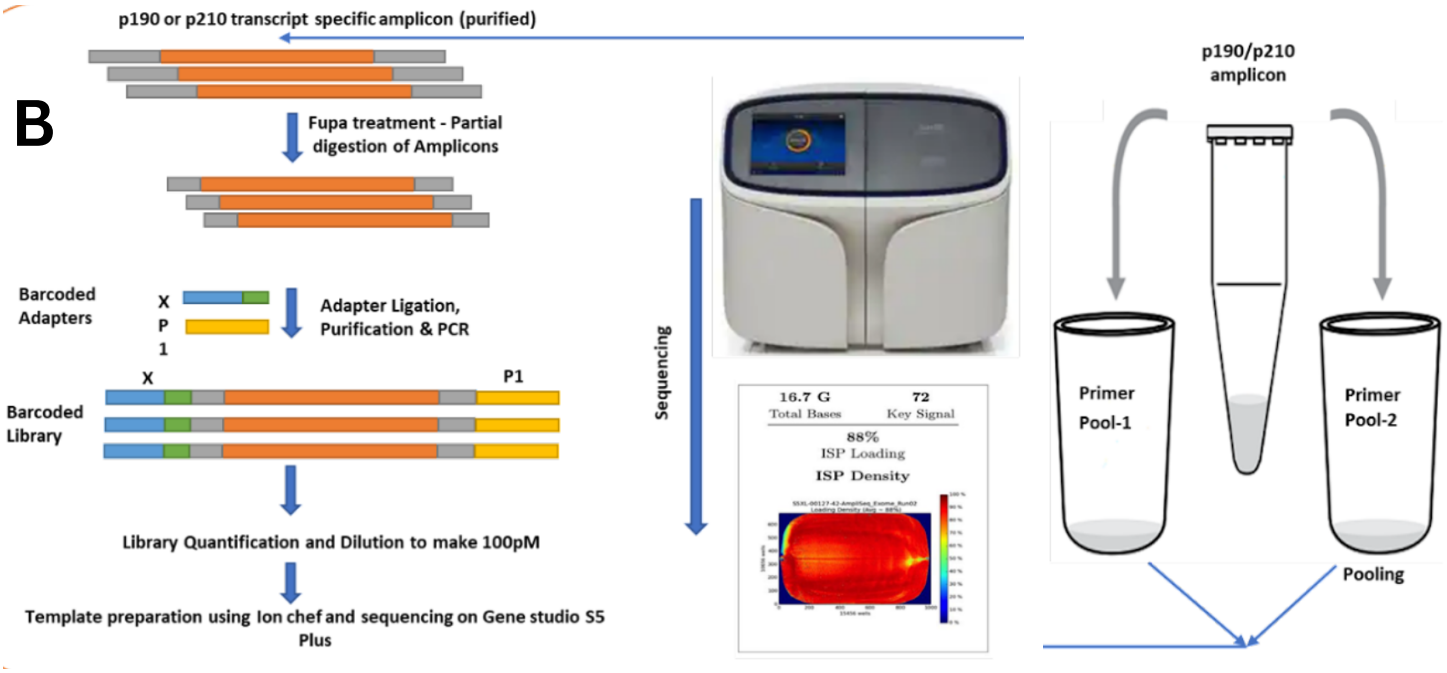


A. Process flow from sample collection to transcript-specific amplification. B. Amplicons were amplified in two separate reactions by two primer pools and pooled together as per the protocol of the Ion AmpliSeqTM Library Kit and sequenced using the Ion Torrent gene studio S5 plus platform.



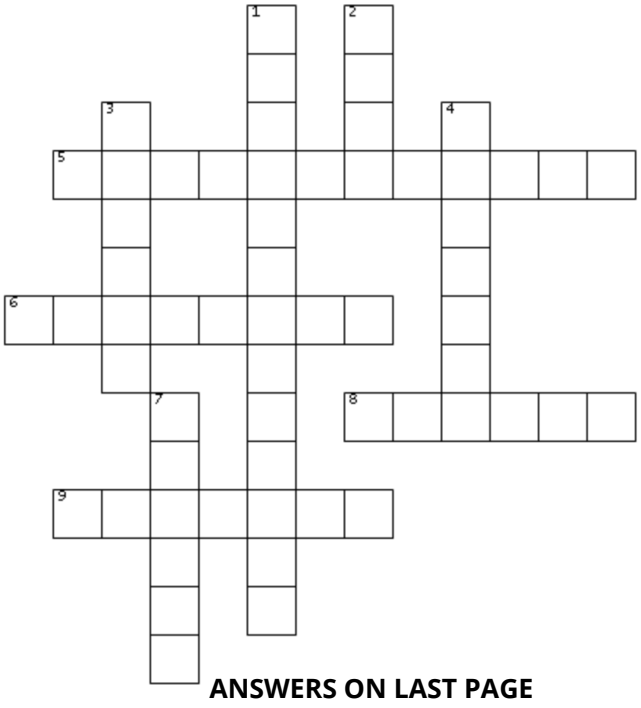
NUTRITION CROSSWORD

ACROSS

5. These seeds have 23% of daily iron intake
6. Green vegetable aiding iron absorption
8. Carbohydrate-rich starchy root vegetable
9. A leafy plant that is high in iron

DOWN

1. The cacao tree's seed product is loaded with antioxidants
2. Dried fruits are high in minerals, vitamins & …
3. Healthier rice alternative
4. Beef, lamb & pork (protein-rich) are classified as…
7. Naturally gluten-free ancient grain rich in micronutrients and protein



ANSWERS ON LAST PAGE

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Prevention of Transfusion Transmitted Infections (TTI): *A Practical Approach*

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INTRODUCTION: *CHALLENGES AND GAPS IN BLOOD SAFETY*

The unavailability of timely, safe blood transfusions remains a significant challenge worldwide, with 42% of donated blood units concentrated in high-income countries, while 16% of the global population resides in these regions. Inadequate screening for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and syphilis contributes to the risk of TTIs.

Low-income areas face additional hurdles due to limited access to high-quality testing kits, further straining healthcare systems. Patients on frequent transfusions or hemodialysis are particularly susceptible to acquiring HCV. The prevalence of transfusion-transmissible infections varies among income countries, highlighting the need for targeted prevention strategies.

Countries	HIV	HBV	HCV	SYPHILIS
High-income	0.002%	0.02%	0.007%	0.02%
Upper middle-income	0.10%	0.29%	0.19%	0.35%
Lower middle-income	0.19%	1.70%	0.38%	0.69%
Low-income	0.70%	2.81%	1.00%	0.90%

Screening Quality Adequacy

The adequacy of screening protocols significantly impacts blood safety. The quality adequacy for different sets of countries is as under.

- 99.8% of the donations are in high-income countries.
- 99.9% in upper-middle-income countries
- 83% in lower-middle-income countries
- 76% in low-income countries.

Characteristics of Agents of TTI

Transfusion-transmissible infectious agents possess specific characteristics that contribute to

their persistence and difficulty in identification during the donor selection process. Presence of the agent in one or more components of blood for long periods. Stability of whole blood and blood components. The long incubation period before the appearance of clinical signs and symptoms. Asymptomatic phase or only mild symptoms in the blood donor, hence not always identifiable during the blood donor selection process.

Viruses	Parasites/ Bacteria/ Others
HIV-1/2, HBV, and HCV	Malaria & Leishmaniasis
HAV & HEV	Babesiosis, Lymes & Chaga's Disease
Hepatitis of unknown origin	Syphilis, Gonorrhoea & Yaws
HTLV-I AND II	Salmonella, Campylobacter
Herpes viruses	Yersinia, Staphylococci, Streptoptococci
Dengue, Chikungunya, West Nile	Mycobacterium tuberculosis
MMR & Chickenpox	Rickettsial agents
Influenza viruses	Prions
Hanta virus	

Screening Protocols and Testing Technologies

Effective screening protocols are crucial for identifying TTIs. Each country has its National policies. Universal recommended testing methods include

- **HIV-1 and HIV-2:** screening for either a combination of HIV antigen-antibody or HIV antibodies.
- **Hepatitis B:** screening for hepatitis B surface antigen (HBsAg)
- **Hepatitis C:** screening for either a combination of HCV antigen-antibody or HCV antibodies
- **Syphilis** (Treponema pallidum): screening for specific treponemal antibodies. Malarial parasites in India

NEWER TECHNOLOGIES: PATHOGEN REDUCTION TECHNIQUES (INTERCEPT)

Challenges in Releasing Blood

- Releasing blood for transfusion presents several challenges.
- The window period of infection or failure due to assay sensitivity or error
- Emerging infections for which screening is not available or effective
- A donor may be infected with an infectious agent for which donations are not routinely screened.
- The donor selection process must be comprehensive. Donor risk assessment is the key protocol for ensuring blood safety.

Recommendations for HBV and COVID-19 Screening

Specific recommendations for HBV and COVID-19 screening are provided to minimize the risk of TTIs. These recommendations involve accepting or deferring blood donation based on factors such as past infection history, current contacts, immunization status, and exposure to infectious agents.

Recommendations for HBV: ACCEPT

- Individuals with a past history of HBV later than 12 months
- Current sexual contacts of individuals with a history of HBV infection if more than 12 months ago
- Current and former household contacts who have been successfully immunized against HBV and are anti-HBs positive more than 100 mIU/ml but anti-HBc negative
- Donors with initially reactive results for HBsAg but confirmed to be non-reactive: re-entry procedures should be established and followed.

Recommendations for HBV: DEFER

- Individuals with active HBV infection or a history of infection within the last 12 months
- Current sexual and household contacts of individuals with active HBV infection
- Former sexual contacts of individuals with active HBV infection: defer for 12 months since the last contact
- Former household contacts of individuals with active HBV infection: defer for 6 months since the last contact
- Health workers who have suffered an inoculation or mucosal injury: defer for 12 months following the exposure; health workers who have been vaccinated against HBV should be assessed individually

Recommendations for COVID-19

Usually, DEFER for 28 days for donor citing a history of travel, contact with an infected case, or recovering from a clinically proven COVID-19 infection.

Pathogen-Reduced Apheresis Platelet Components

The INTERCEPT Blood System, an FDA-approved pathogen reduction technique, offers a promising approach to reducing the risk of TTIs.

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What is pathogen reduction or pathogen inactivation (PI)?

Treating the blood component soon after collection in order to inactivate any remaining infectious agents. chemical agent (Amotosalen) activated by UV-A light and binds to DNA. Prevents DNA replication. Effective against viruses, bacteria, parasites, and protozoa

Benefits of PI

- Materially reduce the risk of TTI.
- Eliminate serologic testing for CMV and production of CMV-reduced-risk components.
- Eliminate the need for irradiation to prevent TA-GVHD.
- Protect recipients from sepsis and Zika virus infection.

Universally adopted technologies are:
Direct detection by microscopy, Serology, Antibody, Antigen, Antigen + Antibody (4th Gen), Rapid tests (Lateral flow, Flow through, ICT), ELISA, CMIA/CLIA
Molecular Methods: NAT, PCR, LAMP

The Final Word

Although advancements in technology provide opportunities to improve blood safety, achieving perfectly safe blood remains a distant goal. Comprehensive and focused donor selection processes, alongside advancements in screening protocols and pathogen reduction techniques, play a crucial role in minimizing the risk of TTIs. Continued efforts are needed to ensure the availability of safe blood transfusions globally.

In conclusion, preventing transfusion-transmitted infections requires a multi-faceted approach involving improved screening protocols, testing technologies, comprehensive donor selection, and the adoption of pathogen reduction techniques. By addressing the challenges and gaps in blood safety, healthcare systems can mitigate the burden of TTIs, safeguard patient health, and enhance the overall quality of transfusion services.

Publications

- Roy P, Sahni AK, Jindamwar P. An urgent need for the introduction of fourth-generation Ag-Ab-based EIA for the detection of HIV infection. Med J Armed Forces India. 2014 Jan;70(1):89-90.
- Roy P, Patel A, Lole K, Gupta RM, Kumar A, Hazra S. Prevalence and genotyping pattern of hepatitis C virus among patients on maintenance hemodialysis at five centers in Pune, India. Med J Armed Forces India. 2019 Jan;75(1):74-80.
- Roy, Partha, et al. Occurrence of false positivity in a fourth generation (Ag/Ab) HIV screening assay: horns of a dilemma. International Journal of Research in Medical Sciences, [S.l.], v. 6, n. 7, p. 2423-2429, june 2018. ISSN 2320-6012.

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Decoding hereditary Cancer: Road to genetic wellness and future health.

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THE ONSET

In the amazing world of our bodies, there are tiny building blocks called cells. These cells possess an intricate dance of growth and division in a controlled way to keep us healthy. They follow instructions that are stored in our DNA, which is like a secret code. But sometimes, this code can have errors, disrupting the harmonious process of cell division and proliferation.

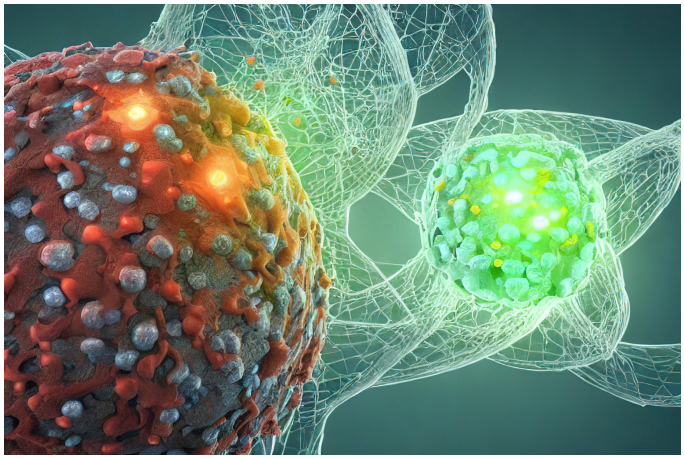
THE CONSEQUENCE?

Unchecked cell growth, is a hallmark of cancer. In this article, we will explore the concept of hereditary and sporadic cancer, understand the genetic changes that cause it, and the role of genetic testing.

Cancer happens when our genes, which are like little instruction books, have changes in them. These changes are called mutations. When genes have mutations, they don't work properly anymore, and that can make the cells grow in an uncontrolled way. Over time, more mutations can happen, and after several years of uncontrolled cellular growth, cancer can develop.

Now, let's explore two types of cancers: sporadic and hereditary. Sporadic cancers arise from genetic mutations that occur after birth, and they are not passed down from our parents. It's like a surprise mutation that is acquired in our body. These acquired mutations do not pass from one generation to the next.

Therefore, if someone has sporadic cancer, their brothers, sisters, or children are not at risk of inheriting the same genetic anomaly. In contrast, hereditary cancers operate by a different set of rules. In these cases, a person inherits a mutated gene from their parents. These genes are like family heirlooms, passed down through generations, placing subsequent offspring at risk of inheriting the same genetic anomaly. Those who possess genes that make them more likely to get cancer have an elevated likelihood of developing specific cancers during their lifetime.



However, it is important to note that not everyone with an inherited cancer susceptibility gene will inevitably develop cancer. ***It is estimated that around 5 to 10 percent of cancers are hereditary in nature.***

To comprehend the inheritance patterns of these genes, let us revisit the basics of biology, akin to revisiting the alphabet we learn in school. We have 26 letters in the English language, right?

Well, our DNA is made up of four special letters called nucleotide bases:

- A (adenine), T (thymine)
- G (guanine), and C (cytosine)

These bases align themselves in varying sequences, just like how letters form words, giving rise to a multitude of genes. Our DNA hosts a repertoire of approximately 20,000 genes.

FATE OF GENES: Accidental or predetermined?

Genes are not something we can buy at a store; genes are inheritable treasures, passed down from both parents. Each one of us possesses two copies of every gene — one inherited from our mother's egg and the other from our father's sperm. When these two genetic contributions unite during fertilization, a new human being emerges, with every cell carrying two copies of inherited genes.

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When an individual inherits one copy of a mutated gene from one parent and one normal copy from the other, they are termed heterozygous or carriers for that particular gene. If this gene happens to be a cancer susceptibility gene, it signifies an increased risk of developing cancer in their lifetime. Each child of such an individual has a 50% chance of inheriting the mutated gene copy, placing them at risk of hereditary cancers. Likewise, they also have a 50% chance of acquiring the normal functioning gene copy, in which case their cancer risk would be no higher than that of the general population.

Additionally, we encounter familial cancers—a distinct category where multiple individuals within a family experience cancer, yet the exact genetic mutation triggering the condition remains unidentified. ***It's like a family mystery waiting to be solved.***

Knowing if cancer is hereditary or sporadic is important because it helps us take the right steps to early detection and potential prevention strategies. Families with hereditary cancer warrant the attention of Genetic Counseling specialists, who conduct pre-test evaluations, assess individual risk for each family member, and offer appropriate genetic testing. At-risk individuals, even if they are unaffected by cancer, can opt for genetic testing, a simple blood test that screens for hereditary cancer markers. Post-test genetic counseling sessions prove invaluable, shedding light on the most suitable preventive and therapeutic approaches.

TESTED POSITIVE FOR CANCER GENES: What should you do if you don't have Cancer yet?

For people who test positive for hereditary cancer genes but don't have cancer yet, there are three main options to address potential health risks.

These options primarily include

- heightened surveillance (keeping a close eye on their health),
- Preventive measures through medications, or
- prophylactic surgery (having surgery to remove the risk).

The Illustration:

Let me illustrate this with an example widely known "Angelina Jolie Effect." After witnessing her mother, grandmother, and aunt succumb to cancer, Angelina Jolie decided to undergo genetic testing. The results revealed a mutation in her BRCA1 gene, which significantly elevates the risk of breast and ovarian cancer.

After thorough consideration and consultations with medical professionals, she chose to undertake prophylactic surgery—a bilateral risk-reducing mastectomy (removal of both breasts) followed by a bilateral salpingo-oophorectomy (removal of both ovaries and fallopian tubes) two years later.

Remember, there exist multiple pathways to address a positive genetic test result. The paramount aspect is to become informed about the available options and make decisions that align with one's personal circumstances.

No decision is inherently right or wrong when it concerns your health. However, I strongly recommend engaging in thoughtful conversations with your healthcare provider to navigate the most appropriate path for your well-being.

That's all for Part 1 of our journey into hereditary cancer. ***Stay tuned for Part 2, where we will delve even deeper into this captivating subject with more fascinating information! Stay curious! Keep questioning and seeking knowledge!***

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FOR ANY SUGGESTIONS OR QUERIES WRITE TO INFO@UNIPATH.IN

ANSWERS TO NUTRITION CROSSWORD

DOWN:
1. DARK CHOCOLATE
2. IRON
3. QUINOA
4. RED MEAT
7. QUINOA

ACROSS:
5. PUMPKIN SEEDS
6. BROCCOLI
8. POTATO
9. SPINACH

FUN FACT

SCARED OF A PHLEBO'S VISIT?

They may take much of your blood when you have a blood test. DON'T WORRY!

15 million blood cells are produced and destroyed in the human body every second!

SERVING YOU SEAMLESSLY THROUGH OUR WIDE PRESENCE

OUR NETWORK