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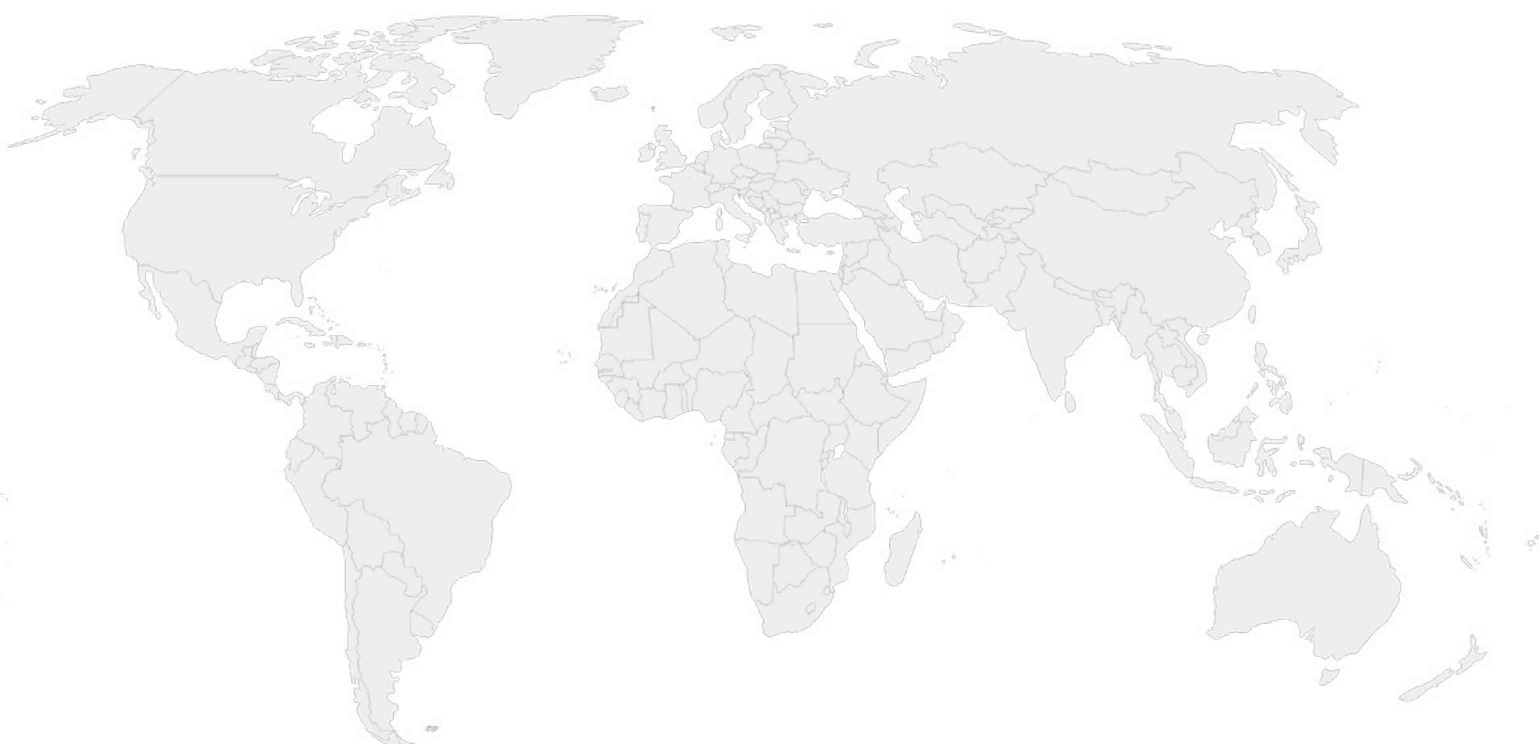

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Letter to the Editor

Point of Care Tests - The Future of Diagnostic Medicine

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POCT, future, diagnostics

Abstract

Dear Editor,

We want to highlight the gargantuan impact that Point of Care Tests (POCTs) have had on diagnostic medicine, and how it will change the landscape of the healthcare system as we know it. POCTs were initiated by Dr. Kost at Davis Medical Centre, California University in the early 1980s [1]. Point of Care Testing (POCT) in the healthcare ecosystem refers to performing rapid, effective, convenient and accurate diagnostic tests near the patient instead of the much more common method of procurement of results via centralized laboratories, which lead to delays in patient care. This can be of particular importance when it comes to time sensitive cases, like sepsis or acute coronary syndrome [2, 3].

One of the most important features of POCTs are their convenience, allowing testing to take place anywhere, from hospitals, clinics, resources limited areas and, in some cases, even a patient's home. This accessibility is extremely useful in rural/ poverty-stricken regions where labs may not be available, ensuring that disparities in the quality of healthcare provided are reduced severely.

Another positive aspect of POCTs are the fact that they are driving innovation when it comes to diagnostic medicine. Examples of this are microfluidic technologies that have been implemented in recent years to check for specific biomarkers, helping to diagnose cancers, tumors and other such mutations (via lateral flow assays) before they have become too widespread in the body to treat [4, 5].

They have also been used in conjunction with biosensors to create self-testing, wearable and fully integrated point of care systems [6]. Successful implementation of such POCT technologies could lead to minimal user intervention during operation to reduce user errors; user-friendly, easy-to-use and simple detection platforms; high diagnostic sensitivity and specificity and immediate clinical assessment.

POCTs were even used to detect infectious diseases like COVID-19, helping minimize turnaround times, enhance patient care, satisfaction and enabling healthcare providers to make real-time decisions [7]. This leads to improved patient management, resource allocation and better utilization of healthcare resources.

The initial investment in POCT devices is substantial, but the long-term benefits like reduced hospital stays, unnecessary procedures, and improved patient outcomes, outweigh the cost. POCTs has some disadvantages, mainly due to the potential for less accurate results compared to traditional laboratory testing mainly due to variable personnel training and control over pre-analytical and analytical phases, which requires stringent oversight by the central laboratory [8]. Hence quality assurance, regulatory compliance, operator training, data management and interpretation of tests by med students and doctors are also among the key factors that need to be addressed to ensure the reliability and integrity of POCT results [9, 10]. Additionally, while POCT offers convenience and accessibility, it should be used in conjunction with standard lab testing, particularly in cases where the assays may not be as efficient or accurate at producing results.
Recent advancements in artificial intelligence (AI) have greatly influenced POCT. For example, AI enabled image analysis and pattern recognition algorithms now allow for quick interpretation of lab reports, increasing the efficiency of this diagnostic modality with optimal quality assurance [11]. The greatest advantage of AI in point-of-care testing will be its capability to conduct essential diagnostic tests reliably and accurately without requiring skilled or trained personnel. This will have a significant impact on community healthcare, with significant harmonization and standardization [12].

In conclusion, Point of Care Testing has and will continue to cause a radical change in diagnostic medicine, offering rapid, convenient, and cost-effective solutions for healthcare delivery. By bringing testing closer to the patient, POCT empowers healthcare providers with timely information to make informed clinical decisions, ultimately improving patient outcomes and enhancing the efficiency of healthcare delivery. As technology continues to evolve and more and more diseases can be diagnosed via these tests, POCT will undoubtedly play an increasingly integral role in shaping the future of diagnostics and transforming the landscape of healthcare.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

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Review Article

Emerging Molecular Technology in Cancer Testing

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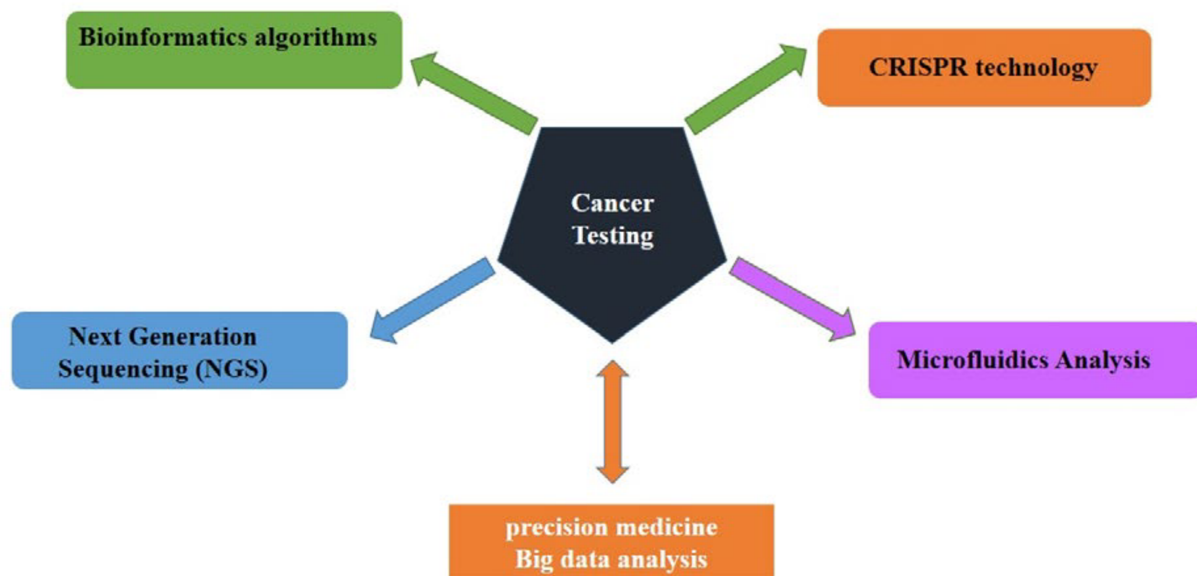
Keywords
genomics, cancer, laboratory medicine and molecular technology

Abstract

Background
One of the areas that have most likely profited from technological and methodological advancements in genomics is cancer research. Genomic technologies are reaching the point where genetic variation in patients can be identified with high precision and at a lower cost, offering the promise of profoundly changing medicine. The next task at hand is to apply the amazing tools and resources created in genomics to improve our knowledge of health and illness. Personalized medicine offers tailored treatment that targets the appropriate drugs for the right person at the right time based on that person's unique profile. This improved understanding should revolutionize the medical sector to leverage genomics in this transformation. Content: Clinical biomarker discovery can be advanced by high-throughput technologies like genomic sequencing and gene expression microarrays. Commercially and at particular academic cancer centers, targeted cancer gene panels (50–250 genes) are offered by Clinical Laboratory Improvement Change-certified laboratories. Precision cancer medicine is becoming a reality thanks to the abundance of data that genome sequencing and other high-throughput technologies provide, both in terms of cost and efficiency. Summary: This review sheds light on the newly emerging molecular technology of diagnostic applications in the clinical laboratory for cancer diagnosis using genomics.

Introduction
The potential of genetic information to ameliorate disease is a great deal of excitement. In the last ten years, genetic technology has become more widespread with tests that help manage drug usage, help prevent and treat cancer, and identify other health hazards (Figure 1) [1]. These advancements show that genomic information will likely be used in clinical care in a large way going forward, going beyond the assessment of single-gene abnormalities to include overall disease vulnerability. Clinical utility, however, becomes a significant problem when the focus moves from highly penetrant genetic diseases to less penetrant genotypes and genetic risk profiles [2]. Precision medicine, which offers individualized medical care based on a patient's specific information and unique genetic profile, is a result of technological advancements. When opposed to the more conventional indiscriminate radio/chemotherapy strategy, this notion has been demonstrated to be effective in improving clinical results [3]. The FDA has approved a rapidly expanding list of medications to treat

Figure 1: Emerging cancer testing methods.



advanced solid tumors that specifically target specific genetic changes. Therefore, it is important to understand and evaluate current and emerging molecular methods for cancer diagnosis and the various molecular techniques available to map the molecular heterogeneity of tumor for effective treatment strategies. Due to the scarcity of nucleic acids in the heterogeneity of tissue and samples, the identification of nucleic acid and Biomarkers was difficult before the invention of Polymerase Chain Reaction (PCR). Using more sensitive quantitative PCR (qPCR) tumor-specific DNA can be amplified to detectable levels [4]. RNA-based biomarkers such as miRNAs can be detected using quantitative reverse transcription-PCR (RT-qPCR) [5]. As technology develops, techniques like qPCR, digital PCR, and NGS can be used to identify exceedingly rare biomarkers like tumor-specific cfDNA. When using qPCR to detect highly rare events or gene variants in a patient sample proves to be challenging, the most recent PCR option to hit the diagnostic market is digital PCR. Conversely, NGS denotes high-throughput nucleic acid sequencing systems that employ PCR-amplified and fragmented DNA at a rate of similar efficiency to digital PCR [6]. This review sheds light on uses of the emerging technologies such as CRISPR, Microfluidic chip-on devices, precision medicine, and NGS big data challenges for cancer.

Novel Cancer Diagnostic Technologies

Recent studies to develop the diagnosis of cancer have broken multiple records for diagnostic test speed and accuracy. Although these molecular diagnostic techniques were first employed as research instruments [7], they have now been demonstrated to be helpful in a therapeutic context [8]. The availability of multiple high-throughput and high-resolution approaches to identify abnormalities in these novel biomarkers has made the use of these biomarkers in cancer diagnosis easier, as Table 1 illustrates.

Table 1: Current high throughput tests for cancer diagnosis.

Analysis	Methods	References
MicroRNA and RNA	Microarray technology	[8]
Methylation analysis	Quantitative sequenom and pyrosequencing	[9]
Single nucleotide polymorphism (SNP) arrays, gene arrangements	Capillary electrophoresis	[11]
Single nucleotide polymorphism genotyping	Matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) sequenome	[12]
70 Gene microarray panel analysis in breast cancer	MammaPrint	[13]
Hotspot cancer mutations	Ampliseq	[14]
Hotspot cancer mutations	Afirma gene profiling	[15]

New platforms, including targeted gene panel sequencing, microarrays, FISH, capillary electrophoresis, real-time PCR-TaqMan assays, nested PCR, sequencing/pyrosequencing, sequencing, and qualitative PCR-ARMS and RFLP, are available for clinical use in cancer diagnosis [9]. These platforms are based on basic, translational, and clinical research [8]. Single nucleotide polymorphism (SNP) detection, gene expression profiling, viral load quantification in cancer, and monitoring, and tracking the outcomes of a patient's treatment are just a few of the many uses for quantitative polymerase chain reaction (PCR), which is widely used in the detection of DNA, RNA, and miRNA abnormalities in the primary diagnosis of cancer. There are several drawbacks of gel electrophoresis, including low resolution, inaccurate results, and non-measurable outcomes. In order to identify gene rearrangements, single nucleotide polymorphisms (SNPs), and loss of heterozygosity (LOH), capillary electrophoresis was created and is now commonly utilized [10]. A panel of more than 46 genes may now be sequenced for a cancer diagnosis in 48 hours for about $1000, thanks to rapid technical advancements and sequencing. As most tests are more accessible and useful than PCR and NGS, qPCR is currently the molecular method of choice for biomarker discovery. Also, test results can be obtained within a day. However, performance is limited to multiple targets and requires prior knowledge of the target DNA. On the other hand, an NGS study can provide valuable information, including mutations, chromosomal rearrangements, and genetic changes, without prior knowledge of the target. Results last about 7 days [11]. Setting up a central and private cancer center as a model for a major city or provincial health center has many challenges. Sensitivity testing is required, which involves sampling from the device during transport, resulting in a longer TAT. Therefore, ill patients may have to wait a day or two weeks for appropriate care [12].

Selection of Molecular platform

The number of biomarkers evaluated in molecular medicine is increasing in clinical practice [13] and advances in precision medicine require caution. Well-behaved cases are rare. The introduction of large-scale sequencing technology (better known as NGS) in molecular diagnostics is an important step towards helping meet these new needs. NGS can be performed using arrays of various sizes that can identify hundreds of genes. The availability of drug studies in clinical research centers requires knowledge of genomic profiling (CGP) of selected patients. CGP may play an important role in future revisions, but it is not a viable method for current revisions [14]. Precision, specificity, and a fast response time are critical when it comes to cancer diagnosis and screening [15]. The qPCR is more widely available than digital PCR and NGS and has a large number of approved assays; it is now the preferred molecular approach for biomarker identification. For instance, hematologic malignancies can be driven by single genetic aberrations that are PCR targets, qPCR is an excellent method for quantifying minimal residual disease. Furthermore, test results may be made available in a single day. However, the activity is restricted to many targets and requires prior knowledge of the target DNA. On the other hand, without prior knowledge of the targets, a single run of NGS can yield significant information, such as mutations, chromosomal rearrangements, and copy number alterations [16], although results can take up to 7 days [6]. Establishing a molecular platform should not be based solely on its superior sensitivity, specificity, or performance. Since advanced molecular laboratories cannot be used for all medical applications, consideration must instead be given to the type of clinical application, cost-effectiveness, and the requirement for greater efficiency. There are a number of drawbacks to setting up a major city or state's central, highly specialized cancer diagnosis laboratory to handle samples from nearby clinical sites. A lengthier TAT is caused by the requirement to handle delicate clinical samples during travel and process samples in batches. Because of this, some really ill patients would have to wait days or even weeks to get the care they need [17].

The Analytical technology selection: NGS versus standard techniques

In the framework of clinical practice, the number of biomarkers evaluated in molecular targeted therapy is constantly raised [18]. Advances in point-of-care diagnostics, where careful standardization of the protocol and assure the procedure selection to optimal execution within clinical needs and frequently with limited biological material available. Molecular diagnostics' advent of massively parallel sequencing techniques, or NGS, is a significant technological advancement to address these emerging clinical requirements. Several sizes of panels that can analyze the tens to hundreds of genes can be used for NGS. A complete genome profile (CGP) in specific patient populations is becoming more and more necessary for clinical research facilities to offer drug-targeted investigations. Although CGP is not a practical strategy in modern clinical practice, it is expected to play a significant role in therapy adaptation in the future [19]. Depending on the number of detectable molecular targets, their complexity, and the proportion of patients with biomarkers approved by regulatory bodies and national and international recommendations, NGS approaches should be used in clinical practice for a subset of advanced malignancies. Thus, typical diagnostic protocols include NGS testing for the identification of biomarkers recognized in clinical practice [20]. The cancers that need to be examined using NGS analysis, comprise ovarian, prostate, lung, and cholangiocarcinoma was much examined. Using NGS technology for these cancers enables the best possible utilization of tissue samples and/or the discovery of recently identified alterations that are not detectable through conventional methods of analysis. All molecular genetic alterations by clinical indication, including point mutations, insertions/ deletions (indels), gene copy number variations (CNVs), and structural rearrangements such as fusions, must be covered by the NGS panels that are used. RNA sequencing offers greater

diagnostic reliability for fusions and NGS panels available in a range of sizes. In clinical practice, panels of just ten biomarkers that are approved are adequate. While entire exome sequencing (WES) is currently less suitable for clinical application, the use of large CGP panels covering hundreds of genes should be permitted within the framework of a clinical trial protocol. When tests could only find one biomarker per study, genomic profiling was first restricted to identifying a small number of identifiable alterations [19]. For a long time, this method has been enough to give details on a drug's sensitivity or resistance to a particular tumor site. Many molecular targets and related medications are now available for a variety of malignancies, and developments of understanding and technology, increase the possibilities of precise and customized treatment. Traditional genetic change analysis techniques do not enable the identification of several biomarkers based on the quantity and time range of biological material used in clinical practice. With a general molecular genetic profile of the tumor, NGS technologies enable even more accurate patient selection that is responsive to targeted therapy. It is presently critical to guarantee that NGS tumor genomic profiling tests, whose significance and proof are acknowledged, are equally accessible to cancer patients across the nation. In fact, based on current knowledge and guidelines created at the national and international levels, the application of these technologies must satisfy appropriateness requirements in regard to tumor type, molecular targets, and accessible medications. The ability to deliver tailored medication, identify a molecularly defined subgroup, and assess the epidemiological impact for each patient are all important factors to take into account. While PCR remains the gold standard for the majority of diagnostics based on nucleic acids [19]. However PCR chemicals are expensive, and the method calls for sophisticated lab equipment and skilled workers [21]. Nonspecific amplification can lead to a reduction in detection specificity, even if isothermal nucleic acid amplification eliminates the necessity for thermocycling species. Additional readouts, such as fluorescence probes, oligo chain displacement probes, or molecular markers, can increase specificity [22].

CRISPR: A Novel Approach to Molecular Diagnosis

Numerous biological applications have made use of Clustered Interspaced Short Palindromic Repeats CRISPR-based diagnostics, most notably the identification of nucleic acid-based biomarkers for infectious and non-infectious diseases as well as the identification of mutations and deletions suggestive of genetic disorders [23]. Furthermore, the method has been modified to identify proteins and other tiny compounds. These unfulfilled demands might be satisfied by diagnostics based on Clustered Interspaced Short Palindromic Repeats (CRISPR) [24]. In order to discover mutations, laboratory procedures such as genotyping single nucleotide polymorphisms (SNPs), heteroduplex analysis (HA), and genome sequencing must be conducted using bench-based methods that do not rely solely on hardware. The SHERLOCK system's demonstration of SNP detection sheds light on the use of CRISPR-based detection in genetic point-of-care mutation screening [25]. It can be coupled and incorporated with current testing technologies to enhance current designs, or with new, free, portable devices to allow for on-site treatment [26]. CRISPR-Cas systems have been adapted for a number of uses to date, including targeted editing of genomes [27], epigenomes [28], and transcripts [29], nucleic acid bioimaging [30], cellular event recording [31], and nucleic acid detection. In general, the quickly expanding field of CRISPR-based diagnostics depends on the programmability, specificity, and user-friendliness of CRISPR technology and seeks to produce point-of-care (POC) assays based on nucleic acids that may be used in standard clinical settings. Managing CRISPR-based diagnostics can help track genetic markers that show response to treatment, like BRAF gene alterations, which are frequently utilized to treat melanoma skin cancer [32]. Furthermore, by identifying cell-free mRNA, CRISPR-based diagnostics can be utilized to track gene expression in real-time across different tissues [33]. Naturally, newly created CRISPR assays need to be validated in clinical trials [34], and the assay's validity needs to be confirmed and upheld during clinical use. Nonetheless, we think that the field of nucleic acid-based detection technology will change due to the quick advancements in CRISPR-based diagnostics. The specificity of CRISPR-based diagnostics may alter the existing requirement for benchtop equipment or genome q sequencing for the detection of genetic alterations such as single nucleotide polymorphisms (SNPs). For instance, scientists swiftly created a SHERLOCK-based test during the Zika virus outbreak to find an SNP linked to prenatal microcephaly in Zika virus patients [35].

Furthermore, findings were obtained using the CRISPR-Chip platform in a few fifteen minutes of use. can determine the removal of two exons linked to Duchenne muscular dystrophy in just fifteen minutes [36]. Due to their extremely small concentrations in serum, cell-free DNA and circulating tumor cells would be difficult to detect without a highly sensitive assay. This is where CRISPR-based assays come into play. While PCR-based diagnostics remains the gold standard today, CRISPR-based diagnostics has advanced quickly since its 2017 launch and offers a number of benefits, including simplicity, speed, and reduced cost. They are perfect for care environments where prompt outcomes can expedite therapy and aid in the containment of infection. There are many more drawbacks to PCR-based diagnostics, including dependable access to personal protective equipment, sample reagents, and nucleic acid extraction. CRISPR-based diagnostics, on the other hand, allow for simple readouts and do not require sophisticated laboratory facilities with benchtop thermocyclers. The development of a single-step diagnostic test that complies with the Clinical Laboratory Modification Act (CLIA) must overcome these obstacles, and portable CRISPR-based diagnostics are expected to transform the clinical diagnosis area in the near future [37]. Modern management systems use artificial intelligence to enhance information retrieval; the classification of this

intelligence modifies the feasibility of sharing [38]. When making judgments through the cloud, some researchers, like Ibrahim and his colleagues, merged their expertise in MI machine learning CRISPR detection based on signals transmitted by radio networks with the Internet of Things (IoT) [39].

Personalized medicine is revolutionizing health care in all therapeutic domains

In recent years, there have been breakthrough technological advancements in cancer medicine. For many years, direct biopsy of the tumor tissue for histological and pathological examination has been the primary method of cancer diagnosis. Recent advances in next-generation DNA sequencing and bioinformatic genomics analysis have brought to light a paradigm change in the field, moving from microscopic histological diagnosis levels to molecular genome levels for the diagnosis of cancer [40]. Variations in patient variability and disease heterogeneity result in variations in drug safety and efficacy. This unpredictability sets off a process of trial and error that doesn't stop until each patient receives a safe and efficient prescription. By using predictive biomarkers to inform therapeutic decisions, personalized medicine aims to do away with trial and error. Many of these tests are currently approved by the US Food and Drug Administration (FDA) and can be categorized as complementary or adjunctive diagnostics [41]. These prognostic tests can be useful in other areas of care, even though they are typically utilized in conjunction with oncology treatment. Oncology has been at the forefront of the creation and expansion of the companion and complementary diagnostics sector [42]. The Dako PD-L1 IHC 28-8 pharmDx was approved by the FDA in 2015 as the first supplementary diagnostic tool for patients with non-small cell lung cancer using the anticancer medication nivolumab [43]. Atezolizumab and nivolumab, two cancer medications, now have expanded indications that include melanoma and urothelial carcinoma, making them complementary diagnoses. About 87% of the companion diagnostics market in North America and 95% in Europe is accounted for by oncology. Oncology is anticipated to continue down the path of personalized therapy, with roughly 60% of medications at the conclusion of clinical development based on biomarker data [44]. Complementary diagnostics for various diseases are also being developed by biopharmaceutical businesses sourced from the oncology field. According to some estimates, non-oncology biomarker analysis is used in about half of the medicines that are presently undergoing phase 3 clinical trials [45]. The use of artificial intelligence among the AACC semi-finalists will not only bring efficient, scalable, and effective solutions to a wide range of health problems but also advance clinical precision medicine. These new technologies use and interpret large volumes of patient data that dramatically increase our understanding of the human at the molecular level. In particular, the Numares AXINON® system uses massive metabolomic datasets to obtain molecular information about different organ systems. In addition to its machine learning and metabolomics platform, Numares has identified constellations of patient metabolites that identify acute kidney transplant rejection. This AI-based approach can facilitate faster medical intervention and better outcomes for those suffering from transplant failure. OncoHost's PROphet also analyzes immunotherapy patients' proteomic signatures to inform individual cancer treatment strategies [46]. Instead of relying on standardized immunotherapy protocols, PROphet informs each patient of a more individualized clinical strategy based on molecular profiles that ideally advance treatment. These exciting new platforms have the potential to change the way we approach clinical testing. They use new technology that can ease the burden on healthcare workers. In addition, these systems facilitate fast, accurate, and personalized testing that can provide better treatment plans for various health problems and thus significantly improve cancer patient outcomes [47].

Utilizing molecular Signature for prediction

Gene expression assays such as Oncotype DX (Genomic Health, Redwood City, CA), MamaPrint (Agedia, Inc., Irvine, CA), and Genomic Grade Index (GGI; Affymetrix, Santa Clara, CA) have recently been made commercially available. These technologies identify a prognostic gene signature to predict response to treatment using real-time PCR or microarray technology. The Amsterdam 70 gene profile or signature, which is based on gene expression profiling, is the basis for the development of the MamaPrint test, which has been authorized by the US Food and Drug Administration [48]. Using a microarray platform, this group of researchers found a predictive signature of 70 genes in patients with node-negative breast cancer who were under 55 years old. Genes related to the cell cycle, invasion, metastasis, angiogenesis, and signaling were included in this signature. The 70-gene prognostic signature was proven to be a robust predictor of distant metastasis-free survival, regardless of adjuvant therapy, tumor size, histological grade, age, and node-positive and node-negative tumors as well as treated and untreated individuals. Another validation was carried out with adjuvant chemotherapy-free node-negative T1-2 breast cancers and compared with standard clinical parameters. An enhanced prognosis for distant metastases and overall survival was demonstrated by a 70-gene profile [49]. In node-negative patients receiving tamoxifen treatment, Oncotype DX, a 21-gene recurrence score (RS) prognostic indicator, forecasts the chance of distant recurrence. Patients with ERþ breast cancer patients [50]. 21 genes out of the 250 clinical genes in the National Surgical Assistance Breast and Bowel Project have their expression levels detected by the study. Researches Formalin-fixed, paraffin-embedded tissues were subjected to real-time RT-PCR, which quantified the expression of 21 genes and computed RS. The patients were categorized into three risk groups: high, medium, and low risk. The National Surgical Adjuvant Breast and Bowel Project validated the 21-gene recurrence score in 675 ERÍ node-negative patients receiving tamoxifen. The results demonstrated that RS corresponded with distant recurrence, time between relapses, and overall survival, regardless of age or tumor

size. Additionally, Oncotype DX testing contains indicators including ER, PR, and HER2 that are frequently employed in diagnosis. In order to reclassify patients with histological grade 2 tumors, the GGI signature was created. This information is useful for making clinical decisions. After examining microarray data from 189 invasive breast cancer cases, Sotiriou et al. found 97 genes that were linked to histological grade; the majority of these genes are involved in the control and proliferation of the cell cycle. There was a difference in the expression of these genes between breast cancers of different grades. The expression pattern of intermediate-grade tumors was comparable to that of low- or high-grade patients. GGI can improve therapy choices and increase the accuracy of tumor classification [51]. Tests for Oncotype DX and GGI have restricted coverage, even if MamaPrint is appropriate for patients who are either hormone receptor-positive or hormone receptor-negative and have either positive or negative lymph node depth. The 21 genes and #40, Oncotype DX and #41 profile, were designed to predict chemotherapy response in cancers that were hormone-positive and lymph node-negative, as well as distant recurrence within ten years. After ten years, 15% of patients with ER-positive, lymph node-negative cancer who were treated with tamoxifen had a distant relapse [52]. Although the introduction of these molecular signatures can improve the clinical management of breast cancer patients, the cost of these tests is relatively high, approximately $4,000 for MamaPrint and $3,500 for Oncotype DX compared to conventional pathology tests such as IHC [53]. An important issue for IHC is the accuracy of pre-analytical factors (ie, fixation duration, type of processing, and type and intensity of antigen used) and post-analytical factors (ie, slide scoring) and the cut-offs used to define positive results. and negative results. Therefore, typical pathological testing was compared with these types of molecular signatures. Knauer et al. found that 80% of tumors classified as grade 1 by traditional methods were classified as having a low-risk prognosis by the MamaPrint test, while 20% showed a high-risk prognosis by the MamaPrint test [54]. Class 3 patients had an index of score of molecular profile by compound diagnostics that varied with patients with other comorbid conditions; 88% of these patients were classified as high risk and 12% as low risk based on the 70-gene profile; the average showed approximately 55% low risk and 45% high risk in MamaPrint [55].

Microfluidics Lab-on-Chip Platform

The only detection system currently approved by the US Food and Drug Administration for the enrichment, detection, and enumeration of CTCs is CellSearch (Menarini Silicon Biosystems Inc., San Diego, CA). This system is based on the expression of epithelium-specific cell adhesion molecule (EpCAM) on the surfaces of epithelial-derived CTCs. An average recovery sensitivity of 85% or higher was observed by Allard et al. In addition to the list, whose underlying biological knowledge and applications are limited to achieve widespread clinical adoption, new approaches have been developed to capture CTCs, including microfluidic platforms such as the CTC-Chip, where CTCs interact with an EpCAM coating, micro columns, Under laminar flow conditions [56, 57]. This positive capture platform option and other such platforms still rely on EpCAM detection, which might not be the most accurate way to define CTCs, even with a more straightforward approach [58]. The application of EpCAM-independent enrichment techniques was pioneered by various research in order to address the problem of CTCs exhibiting an EMT phenotype and perhaps as a result of low negative EpCAM expression. This method was described by Sollier et al. as being used to separate and count CDTCs from the blood of patients with breast (25–51 CTCs/7.5 mL) and lung (23–317 CTCs/7.5 mL) [59]. By thoroughly examining CTCs using these novel techniques, either genomically or molecularly, the molecular diagnostic platform’s promise for several clinical applications can be further realized. Fan et al. used CTCs to diagnose hepatocellular carcinoma with high sensitivity and specificity. They were also able to use CTCs as a real-time parameter for risk prediction and therapy monitoring, which allowed for the early selection of tumor-tailored and effective treatment plans [60]. A sensitive and effective method for assessing prostate CTCs was developed by Miyamoto et al. using microfluidic cell enrichment, and they claimed that this strategy may be useful in guiding treatment decisions for both localized and metastatic prostate cancer [61]. Ilie et al. investigated the expression of the MET biomarker and enriched stage III/IV NSCLC patients using CellSearch and ISET technologies (Rarecells, Paris, France). CellSearch revealed that 83 out of 256 patients (32%), had CTCs. CTCs were detected in 80 out of 106 patients, or 75%, using ISET. 72% of ISET CTCs had MET expression, whereas 65% of patients had positive MET expression in the matching patient tissue (93% concordance). Tissue and CTC MET expression exhibited a substantial positive connection, according to quantitative MET expression analysis using H-score [62]. A worm-based (WB) microfluidic system was created by Zhang et al. to quickly monitor biochemical signals connected to metastasis in a controlled setting. The rate of epithelial-mesenchymal transition is correlated with the risk of cancer metastasis. An effective method for assessing the possibility of metastasis is the creation of an EMT index using extracellular vesicles (EV) produced from tumors. Every epithelial cell and EV formed from mesenchymal cells has to be extracted independently in order to produce an EV-based EMT index [63]. In 2021, Hogyeong Gwak and associates created a special microfluidic instrument to divide two kinds of electric cars. In just 6.7 minutes, they discovered that over 90% of EVs expressing both a mesenchymal marker (CD49f) and an epithelial marker (EpCAM) could be eclectically distributed per 100 µl sample volume [64]. Microfluidic systems are novel approaches to cancer diagnosis and treatment that hold great promise for enhancing therapeutic outcomes. Furthermore, compared to other popular procedures, these techniques are better suited for the diagnosis of cancer [65]. These benefits include lower medication and biological sample consumption, more accurate

spatiotemporal parameters and fluid control in the TME, real-time cell invasion and interaction monitoring, accurate tumor and TME mimicking, and improved environmental control [66]. Additionally, various tumor populations respond to therapy in different ways, which presents a difficulty for medical professionals treating cancer. By preserving cancer cell heterogeneity and serving as the appropriate in vivo TME, in vivo microfluidic technologies help to overcome this obstacle [67]. Furthermore, 3D microfluidic tumor models can impose chemokine gradients and alter cytokine transport for adoptive cell-mediated cancer immunotherapies. Additionally, customized immunotherapy approaches to fight cancer can be found since patient-derived cells can be seeded into microfluidics [68].

Despite these significant benefits, microfluidic devices have certain drawbacks that could influence the study of cancer treatments. A few drawbacks of PDMS, which is frequently utilized in the production of microfluidic devices, include toxicity brought on by the slow release of oligomers and the absorption of molecules. It also takes cell types and matrix compositions that are medically appropriate to mimic the natural TME. To further better capture the physiological complexity of in vivo systems, it is necessary to enhance the current microfluidic devices [69]. Advanced technology and sophisticated production procedures are needed to manufacture micrometric structures. Each microfluidic system must use the right materials depending on its intended use. Mass production and commercialization of microfluidic devices also require high levels of experimental knowledge to enable these systems to be widely used in most nations. It will take time to find solutions to the major problems associated with using laboratories and encouraging the general use of this technology for cancer diagnosis and treatment [70]. Currently, the majority of microfluidic devices are restricted to almost two-dimensional planar forms, and the possibility of creating microfluidic devices using 3D printers is being considered.

Big data in the field of cancer

Even though the big data revolution in biomedicine is still in its infancy, oncology in particular has benefited greatly from it. The quantity of data uncovered by cutting-edge technology has already surpassed Moore's Law, which is the benchmark for the exponential rise in computer processor capacity over the previous 50 years. Just 1% of the digital data collected in all fields up to this point has been evaluated, with over 90% of the data produced in the last two years [71]. This trend is expected to continue in the near future due to the growing need for computer processing power and cloud data storage from the billions of smart devices. Oncology is rapidly becoming digital, just like many other fields, and it already faces comparable difficulties with data integration, quality, sharing, and analysis [72]. "Omics" approaches are typically used to describe large-scale investigations that seek to objectively characterize the full range of biological molecules in a particular tissue or individual. They comprise transcriptomics (spectrum and variants of expressed RNAs), proteomics (expressed proteins and their patterns), genomics (point mutations, copy number variations, single nucleotide polymorphisms), and epigenomics (genome-wide investigation of DNA modifications, e.g., cytosine methylation). isoforms), metabolomics (study of various metabolites), and so forth. Occasionally, these methods result in the identification of a single marker that is medically meaningful, like a causal gene or potential therapeutic target [73]. Nonetheless, a more typical outcome of "omics" research is the creation of intricate molecular profiles. Numerous classifiers based on omics offer the semi-automated capability for differentiating between states, such as cancer and health. The majority of high-throughput research works with datasets in which the quantity of observed features greatly outweighs the number of instances that are examined. For instance, even though expression microarrays may assess over 20,000 genes at once, the number of individuals with various illness features is typically only a few hundred observations at most. In any instance, human intellect is primarily responsible for creating workable hypotheses and analyzing the data because this volume of data cannot be handled in a meaningful way by hand. Many studies are being conducted with the goal of incorporating high-throughput technology into clinical trials [74]. Artificial intelligence is developing at a very rapid pace. Meeting the needs of individual cancer patients requires predictive and repeatable treatment strategies based on models with statistical power to advance knowledge about cancer types, patient characteristics, and clinical experience. The flow of "big data" presents a significant challenge for translational research. Molecular profiles of individual patients can be determined by oncologists with the use of powerful techniques like next-generation sequencing. Precision medicine's advantages must be demonstrated for specific tumors as well as for different cancer kinds and subtypes. Similar to this, cancer immunotherapy can have significant advantages for certain individuals, but finding these people is a key obstacle to its broad use. Information sharing is hampered by a variety of issues, such as the technological difficulties in developing systems that are compatible and the simple pricing structures that encourage data security. Research is needed to understand the relationship between disease and phenotype with strata representing more homogeneous populations. Schadt et al. found that associations between various physiological phenotypes (such as physiological traits) and molecular phenotypes (such as DNA variants, RNA transcript level variations, RNA transcript variants, protein abundance, or metabolite levels) together form a functional unit [75]. All of this could hasten the identification of illness subgroups that may have therapeutic or prognostic implications and aid in the creation of more effective treatment plans. Consequently, phenotypic analysis plays a crucial role in clarifying the molecular and cellular physiology and pathophysiology of networks by offering insights into gene, RNA, or protein groups that form pathways or modules, the failure of which can result in phenotypic consequences. The usefulness of linking phenotypes to characteristics of genetic or cellular networks at the genome

size has been demonstrated by a number of recent research [76]. The new discipline of "Health Knowledge Engineering" aims to use deep phenotypic data to bridge the gap between research and clinical practice, enabling results-based research that informs decision-making in a PM and stratified setting.

Future Directions and Challenges

While the field of complementary and adjunctive diagnostics is now dominated by oncology, advancements in other areas of care are being made possible by the identification of predictive biomarkers and technology advancements. The development of companion and supplementary diagnostics for these polygenic disorders is hindered by gaps in our understanding of the disease's progression and the absence of prognostic biomarkers. The difficulty of collecting samples and the lack of blood biomarkers are additional obstacles. The companion and complementary diagnostics industry is projected to grow at a rate exceeding $7 billion by 2024, despite these obstacles. There is a change in the industry about the "one drug, one test" approach. a paradigm where businesses are currently developing multi biomarker panels and high-throughput devices to evaluate several medications at once. In order to guarantee patient safety, this modification necessitates that regulatory bodies create new guidelines for laboratory testing and diagnosis. Whole transcriptome analysis is a useful technique for examining several genes implicated in the development of breast cancer and finding novel prognostic and prognostic indicators, more sophisticated technologies including epigenetics, proteomics, metabolomics, and next-generation sequencing still need to be developed as follows: i) provide a better understanding of breast tumorigenesis, ii) identify new genetic and epigenetic genes, iii) characterize intratumoral heterogeneity, iv) identify mechanisms of therapy resistance, and v) identify new biomarkers for prognosis and prognosis, resulting in better and more accurate breast cancer monitoring [55]. Quick advancements in novel molecular methods yielded fresh insights into the tumor's biological properties and resulted in a molecular reclassification of breast cancer. New biomarkers for neoplastic invasion, survival, and development are found by these genomic approaches and can be progressively added to clinical trials. Patients with breast cancer are receiving more customized care thanks to the combined advancements in genetics and imaging [77]. The identification of an increasing number of biomarkers is necessary to make treatment decisions due to precision medicine's rapid development. Numerous diagnostic labs are using NGS technology in their clinical practice and research because of this demand, particularly in the public health and academic sectors. Access to NGS test networks plays an increasingly vital role in the application of precision medicine in clinical practice in an era where molecular genetic profiling of cancer acquires an increasingly essential role in treatment decision-making [19]. NGS makes it possible to analyze somatic mutations and RNA profiles of spontaneously occurring malignancies, characterize germline DNA in great detail, analyze microbiomes methodically, and more. New hereditary disorders and molecular targets are being found as a result of the ongoing accumulation of data. cancer therapy, diagnostic markers unique to individual tumors, etc. It is important to realize that it took years for the clinical integration of tests that were comparatively easy to interpret and straightforward, such as EGFR mutation testing or BRCA1/2 analysis, and that many issues are still unresolved to this day. Given that each of the several new potential markers is made up of a variety of uncommon and unique molecular events, it is impossible to foresee how practical medicine will handle such a huge number of markers without individual clinical validation. These developments might need to be taken into account concurrently with clinical trial guidelines, information sharing, and collaboration between laboratory and clinical specialists [78]. In the coming decades, as the incidence of cancer increases in LMICs, improving cancer care will become a growing public health priority. To close the current global cancer effectiveness gap, efforts to increase access to cancer care should be combined with strengthening health infrastructure, including capacity for cancer diagnosis and monitoring. Although innovations in cancer molecular engineering can facilitate this process, they have been underutilized. Going forward, comprehensive applied research, deployment of context-appropriate technology, and continued multidisciplinary investment in molecular cancer diagnosis are key to achieving universal health care and equity in cancer care [79]. This will not be a sudden revolution, not least because the quality and affordability of the new genomic technologies are sufficiently high quality and affordable to be available to the majority of the world's population without quality health care to take care of It is almost certain that the technical problems with the accuracy of the sequence data will soon be resolved; however, this does not apply to problems of interpretation. Although the detailed discussion of interpretive paradigms deserves detailed scientific investigation and thorough discussion among basic scientists, clinicians, and policymakers, it is important to emphasize a few key points.

Conclusion

Molecular changes usually precede the clinical manifestations of the disease, longitudinal measurements combined with clinical phenotyping can identify new diagnostic and therapeutic targets for the disease. Technological advances and cost savings now allow us to obtain much deeper personal multi-omics profiles. Finding connections between molecular markers and disease, as well as which self-layer is disrupted and more informative for each disease, can be accomplished by collecting this data from the same person at different times. Assessing the molecular consequences of specific mutations in genes that encode transcription factors, signaling molecules, and other genes might disclose the regulatory networks and pathways that underlie the disease under investigation and provide potential targets for treatment. These methods may work especially well when applied to fields like cancer, which are still poorly understood.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

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Review Article

History, implementation and current use of the IFCC-IUPAC's Nomenclature for Properties and Units (NPU) terminology in Denmark, Norway and Sweden

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Abstract

Electronic exchange of health care data demands code/terminology systems. In the Scandinavian countries, the IFCC-IUPAC's Nomenclature for Properties and Units (NPU) terminology is used for results in biochemistry, pharmacology, and immunology. Implementation, use and administration of NPU has differed between the countries despite similar health care and lab sectors. In Norway and in one Swedish region NPU – with supplementary SNOMED CT codes is also used for reporting results in microbiology. In Denmark and to some extent in Norway and Sweden NPU is also used for ordering tests. In Norway NPU (as part of NLK) has since 2018 been mandatory in requesting governmental reimbursement for laboratory tests. The numbers of national codes vary considerably (DAN: 303, NOR: 1612, SWE: 415). Furthermore, in Denmark >3500 local codes are used for requisition and to communicate more details with the analytical result than the NPU terminology allows. Also, in Norway the NPU codes are by many lab professionals considered insufficient for communicating all relevant information with results. However, the Norwegian reimbursement system has been a strong motivator for implementing international NPU codes. We find it necessary to add information about "how" a measurement is done to the information about "what" is measured in the laboratory report. Until this is settled otherwise, we suggest an increased pragmatism towards producing national codes including method specific information. Furthermore, we recommend that organisations responsible for classifications have heavy professional participation and decision-making competencies in order to lead and guide implementation and optimal use of the classifications.

Introduction

Electronic exchange of health care data between organisations, regions and countries demands common transmission protocols and agreed code and terminology

systems. Exchange of medical prescriptions has recently been implemented within EU using Health Level Seven (HL7), Fast Health Interoperability Resources (FHIR), which is the current standard transmission protocol for electronic communication of health care data in Europe. Currently it is planned to extend exchange of health care data to cover other types of structured health data, including laboratory test results [1]. Transmission of laboratory test results requires an unambiguous standardization defining what is measured, how it is done and what the test results are.

The Scandinavian countries are among the most digitalized countries in the world [2] including their health care systems which for several years have been virtually fully digitalised with electronic health records (EHR) in hospitals [3,4] and at general practitioners (GP) [5,6] and with electronic laboratory information management systems (LIMS) in all hospital laboratories [5]. Furthermore, as is described in this paper, national health care databases to a varying extent collect health information on every citizen in each country, presenting the data in a secure way to the individual patient and to health care professionals with legal permissions.

This paper describes the implementation and current use of the IFCC-IUPAC's Nomenclature for Properties and Units (NPU) terminology in the Scandinavian countries for electronic exchange of laboratory test results and to some extent also for requisition and reimbursement of laboratory tests. The Scandinavian countries have similar health care systems, laboratory organisations and IT architecture in their health care sectors. However, the implementation, use and administration of the NPU terminology have differed between the countries. The different approaches in the three countries together with common experiences highlight strengths and opportunities as well as weaknesses and shortcomings of the NPU terminology in real life. This forms the basis of some recommendations on how to proceed to reach further harmonization. The descriptions and recommendations are based on the authors' experience and opinions.

The national release centres of the Scandinavian countries were given the possibility to review the paper before publication. A few comments were received from the Swedish release centre and they have been incorporated in the paper.

Historical Background

Clinical chemical tests were introduced in health care 100 years ago [7]. For many years results varied between laboratories due to use of non-standardised methods, which was noted in a paper from 1947 [8]. Since then, the number of different tests and test procedures has grown exponentially, and it became obvious that some form of logical and systematic description of how test results should be reported was necessary. During the 1950s, the Système International d'Unités (SI) was developed containing six base units and a list of coherent units by Bureau International des Poids et Mesures. In the same period, the Danish medical doctors René Dybkær and Kjeld Jørgensen strived to find a systematic and consistent way to describe what was measured in the various clinical laboratory tests and used the SI as a basis for the units. Their work was published 1966 in a small book [9], later referred to as "the Silver book" of the International Union of Pure and Applied Chemistry (IUPAC) [10]. In this book the principles of the Nomenclature for Properties and Units (NPU) were given, and the syntax "System—Component; kind of quantity" - for example "Plasma—Glucose; substance concentration" - was introduced to describe what was measured in a laboratory test.

With the advancements of IT technology from the early 1990s and world wide web, electronic communication between health care organizations became possible. Medical laboratories were among the first to embrace IT technology advances that are now vital in automation of laboratory production. This development emphasized the earlier acknowledged need of a standardized terminology for communication of laboratory tests. Due to the close and similar laboratory traditions, cultures, and ties between the laboratory communities in the Scandinavian countries, the choice of the NPU terminology as a national terminology for communication of some laboratory results in Denmark, Norway, and Sweden, supporting more than 20 million citizens (Figure 1), may not come as a surprise.

NPU and the Release Centres

The NPU terminology was endorsed by the International Federation of Clinical Chemistry (IFCC) [11] and IUPAC in 1995. In the following years, the IFCC-IUPAC Committee-Subcommittee on Nomenclature and Properties and Units (C-SC-NPU) headed by the Danish medical doctor Henrik Olesen, published several recommendations, technical reports, and a user's guide [12]. NPU terminology is an international laboratory terminology with the purpose of providing descriptions (NPU codes) of measurands to present what is being measured in the patient and its values. The NPU codes are established according to following principles [9]:

- Each code has a unique meaning of what is measured representing an in vivo patient property. Methods or description of sample material (e.g., EDTA plasma, serum, lithium-heparin plasma) cannot be included in the codes.
- The terms in the descriptions are internationally defined and traceable to international vocabularies from relevant fields.

The NPU codes are unique and well defined. These characteristics are established by the many stringent rules that exist in the terminology. Each concept used in the NPU code is traceable to an international nomenclature/terminology/classification. Stability of NPU codes over time is another characteristic emphasized by the NPU organisation. However, the importance of this characteristic relative to other characteristics as granularity and coverage is discussed later in this paper.

The administration of the NPU terminology is at present situated at the Danish Health Data Authority. This administration takes on the roles as both the International NPU Release Centre and the Danish National NPU Release Centre [13]. The terminology is

free of use, but establishment of National NPU Release Centres is encouraged for the support of national implementation, translation of English terms into national languages, assignment of National Short Names (trivial name) to NPU codes and to establish national codes (DNK, NOR and SWE codes), when needed to support local laboratories. National codes have the same structure as NPU codes but might not follow the strict rules of the NPU terminology. No coordination of local codes is attempted.

Health Care Laboratory Organisation

Basic demographic data of the Scandinavian countries are given in Figure 1. In Denmark and Sweden, five and 21 regions respectively take responsibility for hospitals, general practitioners (GPs), and medical specialists in private practice. In all three countries, the municipalities have responsibilities for social services, nursing homes, rehabilitation services, etc. The only difference between the countries with respect to health care organisation is that GPs refers to the municipalities in Norway.

Most hospitals in the three countries have biochemistry laboratories, while immunology, microbiology, pharmacology, pathology, and genetics are centralized to varying extent and typically organized into separate departments at larger hospitals. At smaller hospitals, these disciplines may have certain functions within the frames of the biochemistry labs, often supported professionally by specialists from larger hospitals.

In Denmark and Norway, departments of clinical biochemistry are responsible for most phlebotomies in hospitals, while in Sweden the laboratories are not involved in in-house phlebotomies. In all three countries, biochemistry hospital laboratories run outpatient clinics for phlebotomies, as do GPs. In Denmark and Sweden, many GPs also do phlebotomies for tests ordered by hospital departments.

Figure 1: Health demographics (modified figure from [4], approved by the authors).

Norway	
Population size	5.3 million
Annual birth rate	11 per 1000
Average number of births	1.6 per woman
Life expectancy from birth, female	85 years
Life expectancy from birth, female	81 years
Annual death rate	7.7 per 1000
Persons aged 65 years or older	17%

Sweden	
Population size	10 million
Annual birth rate	12 per 1000
Average number of births	1.8 per woman
Life expectancy from birth, female	84 years
Life expectancy from birth, female	81 years
Annual death rate	9.1 per 1000
Persons aged 65 years or older	20%

Denmark	
Population size	5.8 million
Annual birth rate	11 per 1000
Average number of births	1.7 per woman
Life expectancy from birth, female	83 years
Life expectancy from birth, female	79 years
Annual death rate	9.5 per 1000
Persons aged 65 years or older	20%

Private laboratories (ex. Fürst and Unilabs) operate on contract with the regions and are in Denmark mostly used for rarely requested tests, while in Norway they perform a broader repertoire of tests ordered from primary health care.

In Sweden Unilabs and Synlab operate as private laboratory organisations in agreement with the regions. In Denmark and Norway, a certain - primarily microbiological - test repertoire has been centralized to national laboratories (Statens Serum Institute (SSI)) and Folkehelseinstitutet, respectively), while in Sweden specific microbiology laboratories are appointed as "National reference laboratories" for given infectious agents within the "Swedish laboratory network for microbiology" (Svensk laboratorienätverk inom mikrobiologi) [14]. Esoteric tests within microbiology are centralized to the Public Health Agency of Sweden.

NPU in Denmark

Health care IT architecture and communication

Electronic communication of health care data in Denmark was introduced by MedCom [15], which was established in 1994 as a public organization with the mission of facilitating the digital cooperation between authorities, public organizations, private entities, and companies who are all linked to the Danish healthcare sector. MedCom is financed and owned by the Ministry of Health, Danish Regions and The Danish municipalities. With the support of relevant national laboratory societies, MedCom established

three national protocols for communicating laboratory results for pathology, microbiology, and a joint standard for clinical biochemistry and clinical immunology [15].

NPU terminology is applied both for requesting tests and for reporting the results in the joint communication standard (biochemistry and immunology). In the two other communication standards, national derived nomenclatures for reporting results are used: MDS (microbiology) and PatoSnomed (pathology), respectively (Table 1).

From 2003, Danish citizens have had access to their laboratory results through a national e-health portal named Sundhed.dk [15]. The initiative to establish Sundhed.dk came from the Association of County Councils in Denmark, the Ministry of Health and others in 2001. The goals of Sundhed.dk are much broader than giving access to laboratory results, and it includes descriptions of radiological examinations, clinical information and medication. Today, the compiled health care data are considered an E-health record. However, the original part of Sundhed.dk is the system presenting laboratory results. These are divided into pathology, microbiology, and clinical biochemistry/immunology sections, to which the respective laboratories transfer the patient results.

Besides Sundhed.dk, Denmark has since 2006 had a national database for biochemical and immunological requisitions, which following download form the basis for phlebotomies performed at hospitals or at GPs – MedCom request hotel [15]. This allows patients being treated by specialized services at major hospitals, sometimes in other parts of the country, to have samples for biochemical tests drawn by the GP or a local hospital. This calls for a common classification supporting requesting across the country. The NPU terminology is used for this purpose in the absence of an alternative. Due to shortcomings in the NPU terminology for describing concepts used for request like panels, reflex tests, or specified groups of tests, several local (actually regional) codes have been introduced for requesting purposes, and being regional, they are translated, when requests are produced in one region and samples drawn in another.

Table 1: NPU and other terminologies in the Scandinavian countries.

Disciplines	**Denmark**	**Norway**	**Sweden**
Immunology/transfusion	NPU	NPU	NPU
Medical Biochemistry	NPU	NPU	NPU
Medical Genetics	-	-	-
Microbiology	MDS	NPU	NPU
Pathology	SNOMED[1)]	SNOMED[1)]	SNOMED[1)]
Pharmacology	NPU	NPU	NPU
NPU classification	**Denmark**	**Norway**	**Sweden**
Total number of active national and NPU-codes	24763[2)]	10932[3)]	9027[4)]
Number of national codes	303[2)]	1612[3)]	415[4)]
Use of NPU classification			
Test requesting	Yes	Yes	Yes
Reporting results	Yes	Yes	Yes
Economic reimbursement	No	Yes	No

Nomenclature for Properties and Units (NPU), Codes for Microbiology in Denmark (MDS), Systemized Nomenclature of Medicine (SNOMED)

[1)] Different versions of SNOMED are used

[2)] As of December 2023. (LabTerm updated 01.12.2023)

[3)] As of December 2023 (NLK version 78280.70)

[4)] As of November 2023

Implementation and use of NPU terminology

In 2001, the Danish Health Authority recommended the NPU terminology as the national laboratory terminology for reporting laboratory results. This was supported and initially recommended by the Danish Society of Clinical Biochemistry (DSKB). Despite the recommendation of the society, implementation was accompanied by discussions and frustrations among laboratory professionals. As illustrated in Table 2, some quantities have numerous local codes to cover needs of requesting and for separating results of a given quantity, which may not be comparable e.g. Potassium measured in venous plasma, venous serum, venous blood, arterial or capillary blood or by various methods of differing technical quality.

MedCom publish lists of all NPU-codes including national and local codes by the five regions in Denmark and by health care organisations e.g., SSI on their home page [15]. From the lists it can be estimated that just for clinical biochemistry, almost 3600 local codes and 112 DNK-codes were in use during winter 2022/23. Thus, only approximately 1/3 of the exciting national codes (Table 1) were actually used by the regions. These figures do not include local codes used for scientific projects or for reporting results of external quality controls. It can't be excluded that to some extent the vast number of local codes in Denmark might be caused by lack of knowledge of official or national codes or that it just was considered faster to use a local code instead of searching for the correct official or national code.

Local codes are communicated nationwide without any limitations, and from the regional code lists it appears that some local codes from one region are also used in one, or even two, neighbouring regions, thus approaching – unofficial - national codes.

Table 2: Number of codes for 10 common measurands.

Measurand	Denmark			Norway			Sweden		
	I	N	L	I	N	L*	I	N	L
P-Potassium	1	0	19	1	0	-	1	1	?
B-Hemoglobin	1	0	22	0	1	-	1	1	?
P-Sodium	1	0	18	1	0	-	1	0	?
B-Leukocytes	2	0	3	1	0	-	1	0	?
P-Alanine aminotransferases	1	0	0	1	0	-	1	0	?
P-C-Reactive protein	1	0	7	1	1	-	-**	0	?
B-Thrombocytes/Platelets	1	0	8	1	0	-	1	0	?
P-Creatinine	3	0	14	3	0	-	1	0	?
P-Alkaline Phosphatases	1	0	0	1	0	-	1	0	?
P-Albumin	2	0	2	1	1	-	1	1	?

I = International code, N= National code, L = Local code
P=Plasma, B=Blood, I = International code, N= National code, L = Local code
* Laboratories were asked if they used local codes for reporting of results to external systems
** For P—CRP, no data regarding NPU-code usage in Sweden was collected

Administration of NPU terminology

The first official NPU administration in Denmark was established at Rigshospitalet, Copenhagen in 1996. The administration was relocated into the Danish Health Authority in 2002 and is at present sited in the Danish Health Data Authority, which creates new NPU on requests from Danish laboratories or national codes when a national scientific laboratory society makes a recommendation of a definition of a term, e.g. an algorithm which is not covered by NPU codes. National codes have the same structure as NPU codes, but they might use terms that have no internationally accepted definitions.

NPU In Norway

Health care IT architecture and communication

Several IT systems are used both in primary care and in hospitals. For three of the four health regions IT systems in hospitals are separate from systems in primary care. The Central Norway Regional Health Authority (RHA) is in the process of implementing a common IT system for hospitals and primary care centres in this geographical region. To facilitate exchange of information between hospitals and primary care, the government launched the national Summary Care Record (SCR) in 2014. The SCR contains information such as selected journal documents and medication. The Norwegian government also has a national e-health portal [17] where citizens can read their hospital electronic records. As of March 2023, the only included laboratory information presented in this portal and SCR are SARS-Cov2- test results. A new national portal for laboratory and radiology results is under development ("Pasientens prøvesvar"), and this process will also include the introduction of laboratory results into SCR.

Implementation of NPU terminology

Work on a national terminology for reporting of laboratory tests was first initiated by the government in 2004 based on the need for unified definitions for governmental reimbursement [18]. The Norwegian directorate of health decided that the NPU terminology should be used. The first edition of a Norwegian version, "Norsk laboratoriekodeverk" (NLK) was published in January 2012. As demonstrated by the parliamentary proposition "One citizen – One patient journal" (2012), there was in this time also an increased focus on improving the interchange and accessibility of patient information [19]. Thus, as stated by the Directory of eHealth, the primary aim of NLK was to obtain unambiguous communication of both requests and results of laboratory tests [18]. The secondary aim was to obtain a national coding system that could be used for statistical and financial purposes. Somewhat ironically, the mandatory use of NLK in Norway is for the secondary purpose, and not the primary: use of NLK for communication has been nationally recommended since October 2014, whereas use of NLK for making governmental reimbursement claims has been nationally required since 2018.

The standardized use of NLK codes in the XML result message consists of the NLK code itself + the "norsk bruksnavn" ("Norwegian usage name"). The laboratories may also replace NLK codes with local code + local names, whenever the NLK codes do not properly cover the communication needs (for instance due to lack of granularity on method used). Since anatomical collection site or more detailed information about the sample material cannot be described by the NPU terminology, the Directorate of e-health has published supplementary tables for specimen type (specimen material) and specimen source (anatomical location), for use by for instance medical microbiology, to annotate what was collected and from where, respectively. However, the use of these tables are not obligatory and standardized between laboratories, as their proper use is not adequately clarified. Furthermore, free text is accepted. In 2023, the directorate of e-health also introduced a supplementary table for measurement methods.

The Directorate of health has driven the development and implementation of NLK with input from laboratory professions at the Directorate's request. Generally, the laboratory professionals supported the idea of a unified coding system. However, there was also widespread concern directed both at the process, the administration of NLK and more specific issues [20]. Initially, the plan was to include all laboratory disciplines in NLK. However, anatomic pathology was already using a Norwegian version of SNOMED and it was soon decided that pathology should not be included in NLK. Medical genetics did not find the terminology suitable for the field and also declined to be included. However, some genetic analyses that are performed by medical biochemistry, pharmacology and immunology laboratories were included in NLK.

Use of NPU terminology

Even though use of NLK is mandatory in the messages used for governmental reimbursement of laboratory tests, local codes can still be used for requesting and reporting, and later mapped to NLK for reimbursement. NLK currently has 10,932 codes (version 7280.70, January 1, 2024). NPU includes a section for National codes that follow NPU terminology but are not included in the international NPU system. There are currently 1612 NOR codes (Table 1).

Use of NLK for governmental reimbursement

In Norway, laboratory testing in an outpatient setting (testing of patients at GP offices, at hospital outpatient clinics, or under municipal care) is covered financially by national reimbursement from the The Norwegian Health Economics Administration (Helfo). Claims from the laboratories to Helfo are made based on NLK codes [21]. Laboratory testing in an inpatient setting is not covered by Helfo, but by the institutions themselves. However, NLK reimbursement codes and price categories are often used as a basis for billing here also.

Each code in NLK was placed into a price category based on reports of costs from laboratories in Norway (e.g., 13 categories for medical biochemistry). The pricing of several thousand measurands based on cost reports from many laboratories is complicated, and while the overall pricing might be accurate, it is likely that many individual measurands are priced too low or too high. However, this problem probably would exist no matter which classification was used for reimbursement.

Laboratories bill for tests with information specified with patient identification and each performed analysis. This data is collected into a database hosted by the Directorate of health (KUHR database), and anonymized data can be made available upon request. Thus, it is now possible to examine regional variation in the use of laboratory tests [22].

Use of NLK for test requesting and for reporting of results

NLK is recommended for communications of both requesting and reporting results of laboratory tests. No national data on the use of NLK for reporting of results exist. Most results are probably reported with NLK codes, and the compliance with NLK is most likely highest for medical biochemistry.

We contacted representatives of the 21 public health trusts and two private laboratories by e-mail on March 17th 2023, to enquire about the use of NLK and local codes for reporting of the ten most commonly used tests in medical biochemistry. Thirteen laboratories responded. As illustrated in table 2, all laboratories use NLK codes for these measurands. However, some commented that local codes were still in use for communication with some primary care centres with IT systems that were not compatible with NLK codes. In the Central Norway RHA, local codes have been implemented for reporting of results for both hospitals and primary care centres included in the regional IT system, while NLK is used for electronic result reports for primary care centres not using the regional IT system. Laboratories sometimes find the NPU terminology unfit for their clinical needs. In some cases, the national NPU centre has shown some flexibility in instituting

national codes (NOR codes), examples being NOR05091 P-CRP high sensitivity (P—C-reactive protein; mass c.(high sensitivity; proc.) = ? mg/L) as an alternative to NPU19748 P—C-reactive protein; mass c. = ? mg/L, and a national code with the unit g/dL for Haemoglobin.

Administration of NPU terminology

NLK is administered by the Directorate of e-health which is the Norwegian national release centre for NPU codes. The directorate cooperates with the international release centre in Copenhagen. The directorate is also supported by a council of laboratory professionals and councils for each laboratory discipline. Requests for new NLK codes are handled by the national release centre which will check if a relevant code already exists in the NLK or the International NPU system and discuss with the laboratory professionals in the relevant council (-s). New versions of NLK are published 5 times a year and all laboratories must update their IT systems for relevant changes in each version.

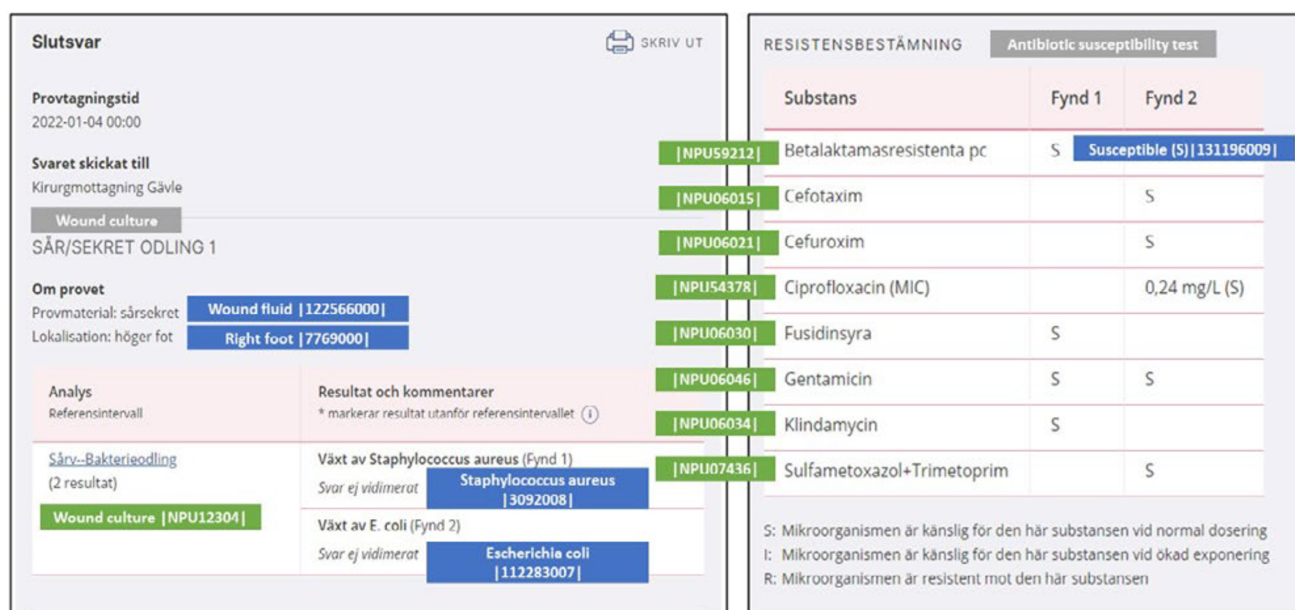
NPU in Sweden

Health care IT architecture and communication

There are at least four different LIMS in use for clinical chemistry in Sweden, e.g., Analytix (CGM), Flexlab (Tieto), LabVantage (Software Point), and Labka II (CSC). To date there are nine different EHR in Sweden. In the next coming years there will be two major EHR, i.e., 17 regions will use Cosmic (Cambio) and two regions Millennium (Cerner). Two regions are about to start a procurement for new EHRs. For primary health care, several smaller systems are in use. Electronic laboratory requests and result reports are often organized specifically for the major systems in the 21 regions, often using local coding schemes for properties measured. The company InfoSolutions provides solutions for electronic communication in other cases.

The national patient overview (NPÖ) initiated in 2014 is a service where health care professionals, with necessary permissions, can view e.g., electronic health care records and laboratory results from different regions and municipalities via the national service platform [23]. The national service platform serves as a switch to enable a safe and efficient information exchange between the health care IT-systems and different national services, e.g., NPÖ. Inera, co-owned by Swedish Association of Local Authorities and Regions (SALAR) and the regions and municipalities, develop and manage the national infrastructure. 1177 journal is the counterpart for citizens to access the information in their health care records from different health care providers, as well as their laboratory results. With these national e-health solutions, it became obvious that a national coding system was necessary for proper identification of laboratory tests, and that the NPU system was an established standard without alternatives for use in Sweden. Since then, Equalis, a company co-owned by the SALAR, the Swedish Medical Society and Swedish Institute of Biomedical Laboratory Science (IBL), with responsibility for External Quality Assessment and also the national release centre for NPU codes, has been engaged together with Inera in the further development of the national information specification for laboratory medicine. The information specification describes how a laboratory result should be structured and coded for exchange of information between the different health care IT systems and the different services (e.g., NPÖ and 1177 journal) via the national service platform. In 2020, the national information specification for exchange of laboratory results was extended to enable the display of results from microbiology, including cultures and susceptibility tests (Figure 2).

Figure 2: Example of a microbiology culture result and susceptibility test in the Swedish National Patient overview (NPÖ).



The green boxes indicate that the message file from the LIMS/HIS include NPU codes for the analyses. SNOMED CT codes can also be included for information about the specimen and the findings. The grey boxes include translations of the headings from Swedish to English in the figure.

There are many patient groups that need to have laboratory tests on a regular basis, e.g., diabetes, rheumatic diseases, and certain types of cancer. Instead of contacting the health care unit or having a meeting with the physician each time, the patient can use the service 1177 "Egen provhantering" and decide when to take the tests and at which health care unit [24]. The physician has then already decided which test that should be taken and how often. To this end, there will be less administration for the health care staff not having to write a referral for tests each time. The patient will also get the results in 1177 journal. NPU-codes are in this case used for both the requests and results. The same service is also used for certain tests that can be ordered by the citizen at any time. A test kit is then sent to the citizen and the test can be taken at home, e.g., chlamydia and gonorrhoea. The test is subsequently sent to the lab and analysed. The results are provided to the citizen in 1177 journal. In this case there is no contact with a health care professional unless the test result is positive. This "testing at home" workflow was also used by many regions during the Covid-19 pandemic for testing of SARS-CoV-2 virus.

Implementation of NPU terminology

The SI was officially introduced in the Swedish health care December 1st, 1975, after a decision by National Board of Health [25] and thereby was also the basic structure of NPU introduced, although without mentioning the term "NPU" which was not coined until later.

No Swedish authority has made any decisions or recommendation about use of a specific terminology. It has therefore been difficult to raise funding for the national management of the NPU terminology. In practice, the cost for national management has been covered by non-paid work and to some extent by the institutions to which these persons happened to be connected. Several investigations into a "National information structure" or framework for the health care system in Sweden have discussed information structure on a general level, without going into details such as which coding system should be used in a common structure and how the management of these coding systems should be funded.

Despite the SFKK recommendation of the NPU coding system, local codes for laboratory tests were still used by the laboratories for a long time, as laboratory results were only locally communicated. There was no central repository of result, for which a common coding system would be required.

The implementation of NPU-codes has been examined within several External Quality Assessment (EQA) programs, where the participants have been asked to report what NPU or SWE-code they are using together with EQA results. Table 1 shows the result for the 10 most common analyses, where 32-48 % reported using a NPU or SWE-code; however, we can't differentiate if not reporting a code means that a local code is used or that a code simply was not reported. This needs to be addressed in a follow-up.

Use of the NPU terminology

NPU codes are primarily used by the laboratories for reporting of results within clinical chemistry, clinical immunology, clinical microbiology, and transfusion medicine. Equivalent to Denmark and Norway, the Swedish NPU database also contains a recommended report name (up to 25 characters) and a short name (up to 20 characters), which are defined by Equalis to make it easier for health care professionals to understand the measurand. The reason for having a short name in addition to the recommended report name, is that some LIMS and EHR systems have a character limitation for the display of names.

The Swedish NPU database currently has 9027 active codes (November 27, 2023) and 415 national codes. There are different reasons why national codes are created, the most frequent being the need a code with the unit percent (%), which represent 148 (36%) of the Swedish national codes.

The use of local codes is only recommended within an organization, however when reporting results externally to e.g., national e-health services or quality registers, NPU-codes should be used. Before the development of the national information specification to enable exchange of microbiology results, it was challenging for the microbiology laboratories to use NPU-codes. The major disadvantage was that the NPU-codes don't describe the specimen type, specimen source, method, and findings. To address this problem, SNOMED CT codes were used as a complement to the NPU-codes, and reference sets were created in SNOMED CT to describe these properties. The combined use of NPU and SNOMED CT codes enabled the microbiology laboratories to code the results in a structured manner, including microbiological cultures and susceptibility tests, and present them in national e-health services (NPÖ and 1177 journal) using the national information specification (Figure 2). The implementation of NPU codes within microbiology has raised new issues by the laboratories in Sweden, e.g., the recommended report names are too short for the microbiology analyses (up to 40 or 50 characters would be needed), and that national guidelines would be necessary to facilitate coding of laboratory orders and results with NPU and SNOMED CT codes.

Administration of NPU terminology

During the period 1980-2000, the Swedish Society for Clinical Chemistry (SFKK) appointed a "nomenclature group" which produced recommendations on how to implement the terminology in various areas. e.g., for excretion of substances in urine and faeces [26]. The group was subsequently joined by Urban Forsum, professor of microbiology, who found the basic principles of NPU well applicable also in the field of microbiology. The nomenclature group made the first translation of the NPU terminology into Swedish, and its use was recommended by SFKK.

In 2000, it was decided to move the management of the NPU terminology to Equalis. The organisation of Equalis, with expert groups in various field of laboratory medicine, was considered well suited for the necessary professional development and

management of the NPU terminology. It was, however, not clear how the work should be funded. A proposal that costs should be shared by the laboratories in relation to their test volume did not work, because the added value of the NPU terminology was hard to see for laboratories that did not yet use the NPU codes for communication of their results. Equalis therefore decided to end the management of the NPU terminology in 2013. After two years of discussions, 21 separate agreements were reached between Equalis and the regions of Sweden that Equalis should resume the management of the NPU terminology, and that the costs would be shared by the regions according to their number of inhabitants. Equalis is now the National Release Centre for the NPU terminology in Sweden.

Discussion

The need for standardization of terminology within health care, including laboratory activities, is beyond discussion and heavily reinforced by the developments in electronic communication, both nationally and - soon - internationally.

In the Scandinavian countries, the NPU terminology was introduced approximately 20 years ago for this purpose, primarily in the fields of medical biochemistry and immunology, though not without problems. The problems can partly be explained by the fact that requesting – although mentioned in The Silver book of IUPAC [9] - and reimbursement is outside the intended scope of the NPU terminology. However, also in the field of reporting results NPU seems to be considered insufficient by many lab professionals.

In our experience many labs want to inform the users of lab results when methodologically different measurements of the same quantity give different results or are of different quality (uncertainty as a consequence of different analytical and pre-analytical variations), via the classification system used for electronic communication of lab results. Thus, many of the Danish local codes are probably caused by the lab professionals wishing to communicate more details of the analytical result to the recipients than the NPU terminology allows for. NPU was developed with the ambition, that when discrepancies between results due to methodological or calibration differences were demonstrated, this would drive development towards standardization and better compliance. This optimistic goal is - despite more than 25 years have elapsed - still not fulfilled, and unfortunately nothing indicates that the goal will be reached in near future. Furthermore, use of local codes for long time periods should be minimized.

In order to achieve this goal, it must be considered whether the possibility of communicating methodological differences of results are optimally met by extending of the NPU terminology as such, by applying supplemental classifications as in Sweden and partly in Norway, or by switching entirely to other laboratory coding systems.NPU is also used for requesting in all three countries.

Since many laboratories receive orders from several different IT systems, a clear definition and uniform use of codes within and between countries would be a great advantage. The extensive use of local codes in Denmark weakens the use of the MedCom request “hotel” (a national database of biochemical requests) across regional borders. Use of NPU codes for requisition of most biochemical quantities function without problems. But anatomical collection site or more detailed information about the sample material cannot be described by the NPU terminology, and NPU cannot express requisition of some more complicated investigations, as e.g. ordering algorithms, microbiologic tests (where specific microorganisms rarely are asked for, rather which pathogenic microorganisms might be present in the actual sample) or make use of synonyms possible, (e.g. commercial names of drugs rather than the pharmacologic names as an ordering option for the clinician). Thus, there is a need for development of the set of codes for ordering, either in national codes or preferably as an international supplement to NPU.

In Sweden, NPU codes are already supplemented by codes from SNOMED CT in order to describe specimen type and source and measurement method. The combined use of NPU and SNOMED CT codes enable the microbiology laboratories to code the results in a structured manner, including microbiological cultures and susceptibility tests. In Norway the Directorate of e-health also has published supplementary tables for specimen type (specimen material), specimen source (anatomical location) and recently also measurement method, and laboratories are encouraged to use the terms as described in the tables. However, it’s our experience that free text descriptions are commonly used, and detailed instructions on how to use the tables is lacking (e.g. how granular should the anatomical reporting be). Furthermore, not all Norwegian laboratories have implemented the use of supplementary tables.

The introduction of funding based on NPU (NLK) in Norway was controversial among many laboratory professionals. However, the previous system was considered outdated and had unclear definitions for the pricing of many tests. According to the Norwegian experience, the dual purpose of using NLK for reimbursement and requesting/reporting has led to a minimal use of local and national codes but also to some unfortunate consequences. Due to technical limitations in the LIMS some laboratories have to report results on all codes for which they claim reimbursement. This leads to “spam” and uninformative test results, simply to trigger charges for the laboratories (e.g., for microbial susceptibility testing, it may be clinically beneficial to report only a few of the tested antibiotics to a clinician, but in order to have the full reimbursement the laboratory may need to report all of them). Second, if laboratories “hunt” for the most profitable NLK codes, this may also impact on their use for communication purposes. If there are two almost similar NLK codes for the same component (e.g., the same component with two different units of measure) the laboratories may choose to report and send reimbursement claims for whatever code has the highest reimbursement category. However, this is not unique for NLK/NPU, and the problem probably would be the same with alternative coding systems, or perhaps even greater with a more

granular coding system than NPU.
Thus, the purpose and/or method of implementation of the NPU terminology in a country seem to have profound effects on the way the terminology is used. In Norway, where NPU serves the purpose of partly funding the laboratories, this has probably contributed to a more strict application to the official NPU-codes also for requesting and reporting. However, local codes are still used in some situations. In Denmark the early introduction of universal electronic communication of both laboratory requests and test results, combined with an administrative habit of using local codes as the solution to many real or experienced shortcomings of the NPU terminology, has led to an extensive use of local codes resulting in a very complicated situation in nationwide IT systems, such as quality databases and Sundhed.dk.
It is a common experience in the Scandinavian countries that introduction of the NPU terminology received criticism by many representatives from laboratories, despite their principal support for the idea of a standardized and international terminology for communicating data. In Norway, the discussion was summarized by Westin et al [20]. In Denmark, the discussions never became as loud and explicit as in Norway, perhaps caused by veneration towards the Danish masterminds of the NPU classification combined with a greater ownership to the terminology compared to Norway, where the implementation of NLK was initiated and driven by central authorities. However, especially when NPU was introduced also for requesting in Denmark, the shortcomings of the terminology became obvious. In the period from 2005 to 2010, a couple of initiatives towards choosing or developing an alternative classification supporting requisition was launched by the Danish health authorities [27], though without success. Could the shortcomings with the implementation of the NPU terminology have been avoided with the LOINC terminology [28], which is used in many countries? The LOINC terminology have many similarities with NPU, from which it originally stems, but also some principal differences [29]. One is that LOINC does not prescribe a specific unit to be used. By using the system Unified Code for Units of Measure (UCUM) to describe the unit, it should be clear for the requester, which unit has been used to express the result from the local laboratory. Another feature of the LOINC system is that the term “System” sometimes is used to denote specimen type instead of the metrological system as in the NPU system. Thus, a measurement of the concentration of free calcium ions in a full blood sample with an ion selective electrode in a blood gas instrument can not be distinguished from measurement of calcium ions in a specimen of serum or heparin-plasma. The NPU terminology provide only a single code, as the quantity intended to be measured is the same regardless of specimen type or measurement technique. On request from users’ information about measurement method and specimen can be included in unique LOINC codes.
This more pragmatic approach within the LOINC system, which allows the user to use multiple units, and to some extent specify sample material and method types within unique LOINC codes, is no doubt appreciated by some users because, at first glance, it can be easier to find a LOINC code, which maps to a local concept. On the other hand, the rapidly increasing number of LOINC codes driven by the need to include increasing non-structured information on specimen and method types, we think will make the system less and less comprehensible. It seems therefore necessary, both for the LOINC and NPU system, to model the necessary information about “how” a measurement is done (such as used specimen type and measurement method) in fields separated from the information about “what” is measured. This is also the way forward suggested within the X-eHealth project [1] for how health care data should be shared in the European Health Data Space. The suggested model will be described in a FHIR profile for the exchange of laboratory data [30].
The main strengths of the NPU terminology are that the NPU codes are unique and unambiguous. These characteristics are important to avoid confusion and miscommunication about laboratory results and, thereby increase patient safety. According to the NPU organisation, codes should also be stable over time. This is emphasized as the main reason for not including method principle/procedure in the codes, as this constantly evolves due to the technological method development. Codes including methodology, calibrators and/or supplier will be used only temporarily, and eventually they will be replaced with other codes. It is argued that this will have impact on the continuity of presentation of laboratory results, and that it will increase the administrative burden for the laboratories.
However, NPU codes have already to some extend been supplemented by national codes (table 1), by supplemental codes in Sweden and by almost 3600 local (actually regional) codes in Denmark, in order to overcome this shortcoming. Thus, the Scandinavian labs are already working with a number of supplemental codes or by high numbers of local NPU codes, suggesting that the argument about stability of codes over time is not accepted by laboratory professionals, who seem to give higher priority to needs of complete information when reporting results or for ensuring correct requesting of tests. However, the cost of this variation of implementation of the code system is lack of comparability of test results over time and between regions.
In summary, it seems the stringent rules that forms the basis of the NPU terminology, and strict adherence to the rules when managing the terminology, defines both the strengths and the deficiencies of the classification system. According to the critics of the NPU classification, it is insufficient in terms of holding the entire amount of data, which laboratories need for requests, and about which they wish to inform the users of test results. Paradoxically, this is caused by deliberate restrictions in the NPU terminology in order to keep the codes stable over time. Thus, in our view, there is a need to identify and implement a pragmatic and functional compromise concerning the extent of the standardization.

Recommendations

The shortcomings of the NPU terminology can theoretically be met either by A) expanding the NPU terminology per se, B) supplementation of the NPU terminology by further classifications holding the information needed for requesting and on analytical methodology, or C) by abandoning NPU and switching to other coding systems (i.e., LOINC), which, however, also needs supplementations. If option B is chosen, we recommend using international classifications, e.g., SNOMED CT for supplementation of the NPU terminology as has been done in Sweden. One of these alternatives must be realized in order to expand electronic communication of health care data from the Scandinavian countries to the international level.

Implementation history of NPU terminology in Scandinavia illustrates the drawbacks of a very rigorous adherence to terminological restrictions. As we find national codes preferable to (numerous) local codes, we recommend pragmatism towards producing national codes in the National Release Centres, even if it sometimes deviates from the principles of the NPU terminology.

Furthermore, the history of implementation of NPU in the Scandinavian countries emphasizes the importance of good terminology governance including heavy lab-professional participation with decision-making competencies to lead and guide implementation of the communication classifications in order to ensure optimal use of the classifications.

Abbreviations

EDIFACT	Electronic Data Interchange for Administration, Commerce and Transport
EHR	Electronic health record
EU	European Union
FHIR	Fast Health Interoperability Resources (an HL7 specification for Health care)
GP	General Practitioner
Helfo	The Norwegian Health Economics Administration
HL7	Health Level Seven
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IU	International Unit
IUPAC	International Union of Pure and Applied Chemistry
LIMS	Laboratory Information Management System
LOINC	Logical Observation Identifiers Names and Codes
NLK	Norsk laboratoriekodeverk
NPU	Nomenclature for Properties and Units
SCR	Summary Care Record (Norway)
SI	the Système International d'Unités
SNOMED CT	Systemized Nomenclature of Medicine – Clinical Terms
SSI	Statens Serum Institut (Denmark)
UCUM	Unified Code for Units of Measure
XML	Extensible Markup Language

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

R. Ceder, K. Toska,, Y.B. Hansen and G. Nordin are, or have been, members of the IFCC committee on Nomenclature, Properties and Units (C-NPU) in collaboration with IUPAC.

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Research Article

A pilot survey on quality control and method evaluation practices in clinical laboratories in Nepal

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Keywords
Internal Quality Control; Method Evaluation; Laboratory Survey

Abstract

Background
There is a lack of systematic collection of information on the quality control practice and method evaluation approach in clinical laboratories in Nepal. Such data is important to formulate educational activities and policy that may address any potential knowledge and practice gap identified.

Method
The pilot survey included twelve questions regarding quality control practice and method evaluation approach and was distributed among the laboratory medicine professionals in Kathmandu, Nepal. Data were collected using a structured self-reported questionnaire on the Google Docs platform. A total of 43 responses were received.

Results
Internal quality control and method evaluation practice varied considerably in terms of the number of levels of material used, frequency of analysis, type and source of material and acceptance criteria among responding laboratories.

Conclusion
The variability in quality control practice and method evaluation approach highlights need for augmentation of knowledge, attitude, and practice behavior among laboratory professionals in Nepal.

Background

Laboratory medicine in Nepal started with the establishment of the first medical laboratory in 1960. After 1990, the private sector began to assume a more significant role in laboratory service provision. As a result, several private laboratories were established throughout the country. Eventually Nepal got its first ISO 15189: 2012 accredited laboratory in 2015[1]. At present the numbers of services and professionals in laboratory medicine have increased. However, the laboratory quality practice and method evaluation approach in Nepal remain areas that requires substantial development. This is in part limited by the lower prioritization for quality and lack of trained laboratory professionals in quality management system given the resource limitation [2]. The clinical laboratory practice is moving toward harmonization globally,

and it is possible to achieve this in a small country like Nepal through the cooperation of clinical laboratories, professional and regulatory agencies, invitro diagnostic industries and metrological institutes. There is currently a lack of systematically collected information about the quality control practice and method evaluation approach in Nepal. Such information is necessary to identify potential knowledge and practice gaps and allow formulation of appropriate educational activities and policy to address them. The goal of this pilot study is to survey the referral clinical laboratories in Kathmandu, Nepal, to identify quality control and method evaluation practices currently in use.

Method

This cross-sectional survey was undertaken in November 2023 among the registered laboratory professionals working in different clinical laboratories in Kathmandu. A structured and self-reported survey questionnaire containing informed consent and other measures was published on the Google Docs platform. Data were collected using the same platform. The respondents provided informed consent for publication of de-identified, aggregated data prior to the start of the survey. Survey of this nature was exempted from ethics approval at the institution where this survey was performed. This study strictly maintained the anonymity and confidentiality of the data. The questionnaire consisted of 12 questions in total. After reviewing the literature in this area and several questionnaires used for an online survey, a questionnaire was designed, and it was reviewed and approved by an independent expert in laboratory medicine. The questions were multiple choices and respondent were allowed to select more than one answer. The questionnaire used in this study was developed for this study. The survey was sent via email to the laboratory personal, representing clinical laboratories in Kathmandu, who registered themselves for a workshop focusing on quality control. There were 48 recipients resulting in 43 responses received. Data were summarized using descriptive statistics and all calculations were done using Microsoft® Excel® 2019. Subsequently a one-day workshop was organized, and survey results were summarized and presented. Additionally, the comments made by the laboratories about their practices in the local setting were qualitatively recorded and presented below.

Results

The survey contained questions on internal quality control and method evaluation. There were 43 responses where 27 were from private standalone tertiary laboratories including nationally recognized accredited laboratories, six were from medical colleges and ten were hospital based laboratories. There was no participation from government laboratories. The individual questions along with the findings of this survey are discussed below. The number of answers is not equal to the number of respondents because option for choosing multiple answers was provided. Thus, the resulting percentage of answers can exceed 100%.

Section 1. Quality Control Practice

1. **Which of the following QC material is used in your laboratory?**

A. Quality control material from reagent manufacturer
B. Quality control material different from reagent manufacturer (third-party)
C. Leftover patient samples
D. Others

Results

All the participating laboratories are using QC material in one way or other. Most of them, 51%, are using QC material from reagent manufacturers as well as third party QC material. Around 10% and 35% of laboratories are using QC material from reagent manufacturers and third party QC material, respectively.

Table 1: Results for the type of QC material used in clinical laboratories in Nepal.

QC Material	Number	Percentage of laboratories
From Reagent Manufacturer	28	65.1
Different from Reagent Manufacturer	38	88.4
Leftover Patient sample	1	2.3
Other	1	2.3

Comments

The use of QC materials from the reagent manufacturer is suboptimal since they may be produced under the same condition and may mask changes in the analytical performance. The use of retained patient samples provides a cost-effective alternative and is considered generally commutable, although the retained samples should be kept in a condition that ensures their integrity and the target values and control limits need to be established by the laboratory.

Recommendation

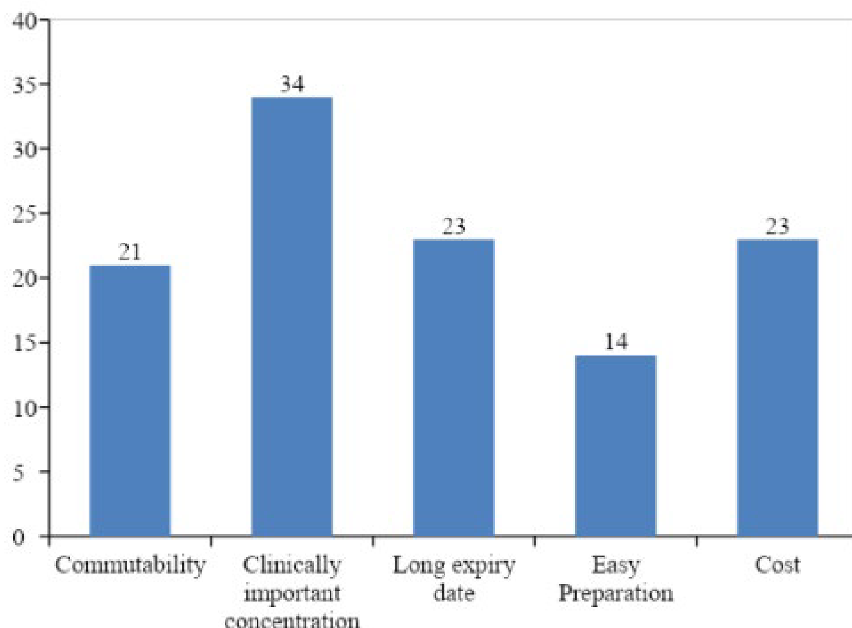
The ISO 15189:2022 standard recommends QC material independent of the assay manufacturer to control the risk of non-detection of drift when changing reagent lot. For some tests, laboratories may have limited alternatives to QC material from the assay manufacturer. In these circumstances, the use of alternate/ additional forms of QC should be considered, e.g. retained patients sample or patient-based real time quality control.

2. What are your main considerations when selecting quality control materials?

A. Commutability of the quality control material
B. Quality control material covering clinically important concentration
C. Long expiry date
D. Easy preparation
E. Cost

Results
Around 20% of participating laboratories consider all considerations equally important. A majority of laboratories primarily consider the availability of QC material at clinically important concentration while the ease of material preparation is least likely to be a main consideration.

Figure 1: Factors considered while selecting QC material (X-axis) versus number of laboratories (Y-axis).



Comments
All factors listed above are important considerations when selecting QC materials. The prioritization of the specific considerations will depend on local circumstances. The emphasis on clinically important concentrations suggests an appreciation for monitoring the performance of the assay at these high-risk concentrations. Having a long expiry and low cost of QC material are operational and financial consideration of equal importance to the survey respondents. Commutability status of a QC material is often uncertain, it is nevertheless important to use QC materials with appropriate matrix for the sample types encountered clinically. Ease of QC material preparation is generally a compromise the laboratory is most willing to make.

Recommendation
National regulatory body should encourage local proficiency testing providers to provide affordable QC materials. It is recommended that laboratories use QC materials that are commutable and covering clinically important concentrations that are within the limits of the analytical measuring range of the assay, without dilution.

3. In general, how many concentrations (levels) of quality control material are used in your laboratory for each assay?
A. One concentration
B. Two concentrations
C. Three or more concentrations

Results
Almost 50% of laboratories use two levels of QC. Just over a quarter (28%) of laboratories uses three or more concentrations. One in ten (10%) laboratories use one level of QC for analytes with low volume of requests and two levels of concentration for those with high volume of requests. A minority (~5%) of laboratories use only one level of QC.

Comments

The surveyed laboratories refer to the various sources the number of level of QC used, including National Accreditation Board for Testing and Calibration Laboratories (NABL), India (Document 112) and peer reviewed literature. Most of the laboratories use at least two levels of QC, which meet the regulatory or accreditation requirements.

Recommendation

Experts in laboratory medicine of Nepal in collaboration with national regulatory body and Accreditation Education Research & Scientific Services Center (AERSSC), an accreditation body in Nepal, should establish a consensus on the number of level of QC that are appropriate for the local laboratory practice.

4. What are the desired concentrations of quality control in your laboratory?

A. Near the lower limit of reporting/ functional sensitivity of the assay
B. Near the reference intervals/ medical decision limits
C. Near mid-point of the assay range
D. Near the upper limit of assay range

Table 2: Results for the number of QC levels used in clinical laboratories in Nepal.

Number of QC levels	Number	Percentage of laboratories
One concentration	6	14
Two concentrations	29	67.4
Three or more concentrations	17	39.5
Other	1	2.3

Results

Only 35% of laboratories use QC materials with concentrations covering the reference interval or medical decision limit for the analyte. The other laboratories consider a mix of functional sensitivity, midpoint, lower and upper limit of assay as the desirable concentrations to be monitored by the QC.

Comments

While having QC material covering clinically important concentrations is the most commonly cited emphasis when selecting QC materials (see Question 2), there is a lack of consensus on what constitute desirable concentration. Most laboratories did not consider reference limits or medical decision limits as desirable concentrations to be monitored by QC.

Recommendations

Choosing QC material with analyte levels which are close to the reference limit and/ or medical decision limit is recommended. These are the concentrations that are liable to affect clinical interpretation of the laboratory results should there be a change in analytical performance. Ideally, at least one QC concentration should cover the reference limit/ medical decision limit while another may cover concentration within the pathological range for the analyte.

5. How does your laboratory establish the quality control target value and control limits?

A. Use manufacturer's target value and control limits
B. Establish in-house target value and control limits

Table 3: Results for the concentration of QC material used in clinical laboratories in Nepal.

Concentration of QC	Number	Percentage of laboratories
Near the lower limit of reporting/ functional sensitivity of the assay	7	16.3
Near the reference intervals/ medical decision limits	22	51.2
Near mid-point of the assay range	18	41.9
Near the upper limit of assay range	16	37.2

Result

Most of the laboratories use QC manufacturer's target value and control limits (82%) while the others established these parameters in-house.

Comment

Laboratories using manufacturer's target value and control limits may do so for a combination of reasons including unfamiliarity with the procedure to derive these parameters themselves, a lack of resources or for convenience.

Recommendation

Target values and control limits provided by manufacturers are often wider than those found within the laboratory. This may

lead to overly lenient control limits, which can compromise the detection of analytical errors. Laboratories should establish its own target value and control limits for QC material using long-term data. The target value and control limits should be reviewed periodically and judiciously adjusted where appropriate to ensure optimal error detection performance.

6. How often is quality control testing performed in your laboratory?

A. Once a day
B. Twice a day
C. Three times a day
D. Before running a batch of samples
E. After running a batch of samples
F. Before and after running a batch of samples

Results
Around 60% of laboratories perform QC testing once a day. Only 18% perform QC testing twice a day. 5% of them run QC once or twice a day depending upon the number of test request for the analyte.

Comments
Laboratories testing QC once a day may do so due to operational (e.g. low test request) or financial reasons. In laboratories analyzing large number of clinical samples between QC testing, there is an increased risk of missed error.

Recommendation
The frequency of QC testing should consider the stability of the analytical performance of the analyzer and method along with reagents, the workload and frequency of the assay and the risk of harm to patients from an erroneous result. It is important to consider testing QC sample before (and ideally, after) patient sample analysis as well as following daily maintenance, calibration and any troubleshooting procedures.

7. Which of the following quality control interpretative rules are being used in your laboratory?
A. 1:2S (1 QC result outside of 2SD)
B. 2:2S(2 consecutive QC results outside of 2SD)
C. 1:3S (1 QC result outside of 3SD)
D. 4:1S (4 consecutive QC outside of1SD)
E. 10x̄ (10 consecutive QC results to the same side of mean)

Table 4: Results for the frequency of QC testing in clinical laboratories in Nepal.

Frequency of QC	Number	Percentage of laboratories
Once a day	32	74.4
Twice a day	10	23.3
Three times a day	2	4.7
Before running a batch of samples	5	11.6

Results
Only 14% of laboratories are using all the interpretative rules for acceptance of QC results. However, all laboratories are using at least one QC rule. The most commonly applied QC rule is 2:2S followed by 1:3S,1:2S and 10x̄ respectively.

Comments
Among the survey participants, nearly all are using Levy-Jennings chart for reviewing the QC results. The 4:1S and 10x̄ rules are less commonly applied and are helpful for detecting systematic changes (bias).

Recommendations
It is recommended that laboratories establish a policy for interpreting QC results, including the setting of QC rules. The 1:2S rule is generally regarded as a warning rule and is associated with approximately 5% false flagging/ alarm rate. On the other hand, the 1:3S and 2:2S are generally considered a rejection rule since these are associated with <0.5% false alarm rate. The other QC rules, such as 4:1S and 10x̄ rules may be considered if systematic error is suspected. It is useful to periodically review the QC data and QC rules to ensure optimal error detection.

8. Do you customize the quality control interpretative rules for different assays?

A. No, we use the same rules for every assay
B. Yes, we customize the rules according to the analytical performance of the assay

Table 4: Results for the QC interpretative rules used in clinical laboratories in Nepal.

QC interpretative rules	Number	Percentage of laboratories
1:2S	20	46.6
2:2S	24	55.8
1:3S	23	53.5
4:1S	7	16.3
10$\bar{x}$	17	39.5

Results

70% of the participating laboratories use the same interpretative rules for all the assays while the other tailor the QC rules according to the number of test request for the analyte.

Comments

Ideally, QC interpretative rules should be tailored such that true errors are detected with minimal false rejections. Most of the laboratories use the same rules for all the assays which may be due to lack of familiarity with QC rule customization or for operational convenience by using standardized QC rules within the laboratory.

Recommendation

When QC rules are tailored according to the clinical requirement, risk tolerance and analytical performance of a laboratory method, it can optimize error detection while reducing false alarm rates. At the same time, the use of tailored QC rule for each assay may introduce significant operational complexity since it may require different frequency of QC testing, different troubleshooting protocol and different QC interpretative rules. Therefore, there is a need of increased resource requirements, the need for advanced data management systems, and the necessity for additional staff training. Phased implementation, leveraging automated systems, and seeking expert consultation would help. Care should be exercised to balance all the above factors when determining the QC policy for the laboratory.

9. Do you use other methods for monitoring the performance of your assay?

A. Yes
B. No

Results

All the participating laboratories only use QC for monitoring the performance of the assays.

Comment

Internal QC remains the only means of monitoring the performance of the assays in the laboratory in Nepal. Patient-based quality (PBQC) is not practiced.

Recommendation

The use of PBQC is a valuable tool for monitoring the performance of the analytical performance of the assay. However, it requires suitable software (instrument, middleware or laboratory information system) to perform this monitoring in real time. In the absence of such advanced laboratory software, common statistical software such as Microsoft Excel may be used to analyze the patient data in a retrospective manner. The use of patient result for monitoring assay may represent a cost-effective alternative for laboratories in Nepal.

Section 2. Method Evaluation

10. Does your laboratory perform method evaluation for a new assay?

A. Yes, before starting clinical service
B. Yes, after starting clinical service
C. No, we do not perform method valuation

Results

Around 88% of laboratories perform method evaluation for a new assay before committing to clinical service. Approximately 10% of laboratories participated in survey do not perform any pre-implementation method evaluation.

Comment

Method evaluation is important for objectively assessing the performance of the laboratory method against manufacturer claims. Data obtained from method evaluation can inform other aspects of laboratory practice (e.g., QC policy). Pre-implementation method evaluation also provides the laboratory with the opportunity to resolve any installation or commissioning issues that may compromise the analytical performance of the laboratory method.

Recommendation

It is necessary to perform method evaluation before committing a new laboratory method to clinical service to ensure it meets the clinical requirement and manufacturer's claim. Method evaluation should be performed with thoughtful planning and execution to ensure laboratory obtains the most useful data for a given amount of resource. Financial constraints in Nepal often necessitates scaled down method evaluation protocols.

11. Which of the following components are routinely evaluated for a new assay in your laboratory?

A. Precision
B. Bias/ accuracy
C. Linearity
D. Method comparison (either with the 'old' assay or a reference assay)
E. Analytical measurement range/ dilution factor
F. Carry-over contamination
G. Assay interference

Results

A majority of laboratories (60%) verify precision, bias and linearity. A quarter of laboratories verify precision and bias. Only 5% of laboratories verify all the components listed in the question.

Comments

When performing method evaluation, the majority of laboratories adopt guidelines that are at least in part developed internally rather than just following international guidelines. There may be financial reason for using the local guidelines, which are often simplified. The lack of local regulatory requirements may contribute to the lower number of analytical components being verified by the participating laboratories. Verifying all the listed performance component can be cost prohibitive to the local laboratories.

Recommendation

There are many components to method evaluation, and each requires dedicated consideration for the experimental design, acceptance criteria and statistical analysis. The component and approach of method evaluation may be guided by clinical requirements, local resources, and local regulatory requirements. The local national regulatory body may provide guidance in this aspect of laboratory practice. At minimum, a laboratory should consider verifying the precision, bias/ accuracy, and linearity of a new laboratory method.

Table 6: Results for the method comparison components used in clinical laboratories in Nepal.

Components evaluated for a new assay	Number	Percentage of laboratories
Precision	41	95.3
Bias/ accuracy	30	69.8
Linearity	34	79.1
Method comparison	17	39.5
Analytical measurement range	7	16.3
Carry-over contamination	5	11.6
Assay interference	4	9.3

12. What material is routinely used for method evaluation in your laboratory?

A. Quality control material from reagent manufacturer
B. Quality control material (third-party) different from reagent manufacturer
C. Leftover patient samples
D. Others

Results

A third (32%) of laboratories use quality control material from reagent manufacturer for method evaluation while a quarter of laboratories use third party QC materials. Only one laboratory uses patient sample for the verification.

Comments

Most of the laboratories use QC material for method evaluation as it is generally accessible, easy to use, and overcomes the challenge of preparing and storing a large quantity of patient samples.

Recommendation

It is ideal to perform method evaluation using leftover patient samples since it avoids potential non-commutability that may be present in QC materials. However, the need to prepare and store these samples in sufficient volume for the method evaluation experiments and in a manner that retains its integrity can be challenging. Moreover, the use of patient samples requires the establishment of target values and uncertainty that may add to the costs. Using QC material for method evaluation is a pragmatic alternative but laboratories should be mindful of potential non-commutability, lack of sample at specific/ desired concentration. Proficiency testing materials can be an alternative.

Table 7: Results for the material used for method evaluation in clinical laboratories in Nepal.

Material used for method evaluation	Number	Percentage of laboratories
Quality control material from reagent manufacturer	29	67.4
Quality control material (third-party)	26	60.5
Leftover patient samples	8	18.6

Discussion

This pilot study surveys the laboratories in Kathmandu, Nepal, on their quality control and method evaluation practices. In this survey, the participating laboratories reported QC practice including the number of levels of material used, frequency of analysis, type and source of material and acceptance criteria. Variability in findings may be in part due to the difference in perceived importance for different considerations for QC practice. Similarly, the method evaluation practice in Nepal also varies among laboratories. The subscription to accreditation may encourage more standardized practice and set minimum requirements. However, only a few laboratories in Nepal are accredited to the ISO15189 standards, which may explain the heterogeneity in practice. Since accreditation may be considered costly, local regulatory/ professional body may consider incorporating some of the accreditation requirements and provide more specific guidance in these areas, taking into account local context and resource availability. The present survey lacks comparative analysis with the international standards. Future follow up studies will include benchmarking against quality control practices and methodologies from clinical laboratories in other countries, particularly those with well-established standards and practices. The interplay between internal QC and external quality assurance (EQA) programs is essential for ensuring the highest standards of quality in clinical laboratories. Internal QC practices are crucial for the daily monitoring and immediate validation of laboratory results, allowing for the detection and correction of errors in real-time. Conversely, EQA programs provide an external benchmark, enabling laboratories to assess their performance against national or international standards and identify areas for improvement. Recognizing the importance of internal QC and EQA programs, further surveys or studies should also focus on EQA programs. The QC practice of a laboratory is influenced by the knowledge, attitude, and practice behavior of its laboratory personnel. A recent study on knowledge of QC practice among laboratory personnel in a medical college in eastern Nepal revealed that only 25% had adequate knowledge [3]. The results of this survey corroborate with the previous survey and provide further evidence that there is a general need to improve the training and education of laboratory personnel in Nepal. The education efforts will help raise the laboratory practice of Nepalese laboratories and improve harmonization of QC and method evaluation practices. Another limiting factor for laboratory management in Nepal is financial constraints. Lack of QC (number of sample and frequency) will mean reduced power of detecting error and increasing the risk of undetected error. This might affect patient care if clinically important error goes undetected. Also, the lack of appropriate sufficient method evaluation may allow method with suboptimal performance to go into routine practice. This may then manifest as poor QC performance, or worse still, if goes undetected, may affect patient results. Ideally, adequate budget should be allowed for appropriate QC testing, participation in external quality assurance programs, pre-implementation method evaluation, laboratory staff training, fee for auditing and accreditation bodies etc. However, the competing financial priorities in a resource constraint setting may mean that some of these considerations are relegated or neglected. There are Nepalese national regulatory policies for total quality management in laboratory. Nonetheless, the implementation of these requirements has been haphazard due to a combination of factors including inadequate knowledge, inadequate guidance, insufficient resources, and poor enforcement [2]. Laboratories may design method evaluation protocols that uses minimal sample size (depending on local resources)/ protocols with suitable statistics to perform method evaluation in resource limited setting [4,5,6]. At minimum, laboratories are advised to evaluate analytical precision, accuracy and linearity. Other specific difficulties encountered by the clinical laboratories in Nepal are shortage of skilled manpower [7], regulatory guidance [8], infrastructure and technological limitation [9], and logistics and supply chain problem [10]. These difficulties can be addressed by policy intervention, capacity building and international collaborations. A major limitation of this study is the inclusion of relatively low number of laboratories that is geographically focused in the capital city of Kathmandu, Nepal as well as the lack of response from government laboratories. However, it should be noted that only the clinical laboratories in Kathmandu were selected in this study due to its representative urban healthcare infrastructure and higher accessibility to diverse laboratory settings. Although this study is based on small sample size, it is still the most comprehensive survey of Nepal to date. Care should be exercise when interpreting the results of this study as it may not be generalizable to other parts of the country that may have different local context (e.g., remote regions). We emphasize that, there is a need of conducting similar studies in diverse geographic locations across Nepal to obtain a more comprehensive understanding of clinical laboratory practices nationwide.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of

interest with regard to the content of this manuscript.

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Research Article

Characterizing Monoclonal Gammopathies in an East Moroccan Population: University Hospital Findings

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Abstract

Background

Monoclonal gammopathies (MG) are frequent, especially among older people. This study aims to establish the features and etiologies of MG detected over seven years in the Biochemistry department of Mohammed VI University Hospital in Morocco.

Methods

The study was performed from Jan 1, 2016, to Sept 1, 2023, and involved 224 patients residing in east Morocco. The diagnosis of MG was conducted through capillary zone electrophoresis, followed by confirmation through immunofixation.

Results

The study included 224 patients, with an average age at diagnosis of 65.91 years. There were 122 (54.46%) males and 102 (45.54%) females, for a sex ratio of 1.19. In terms of immunoglobulin isotypes, IgG was found to be the most common monoclonal protein (59.82%), followed by IgA (19.64%) and IgM (6.71%). Furthermore, 11.6% of cases had exclusive free light chain (FLC) secretion, and 2.23% had biclonal gammopathy. The distribution of diagnoses in our study included multiple myeloma (MM) (78.57%), lymphoma (5.35%), plasma cell leukemia (4.02%), Waldenström macroglobulinemia (WM) (3.57%), and MGUS (1.79%).

Conclusions

Our study noted the high frequency of MM over MGUS. Several factors could contribute to this prevalence, including variations in healthcare access, demographic characteristics, and potentially other elements that warrant further investigation.

Abbreviations

AL amyloidosis: light chain amyloidosis; CRP: C-reactive protein; FLC: free light chains; IQR: interquartile range; LDH: lactate dehydrogenase; MG: monoclonal gammopathies; MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma; sFLC: Serum free light chain; SIFE: serum immunofixation electrophoresis; SMM: smoldering multiple myeloma; SPE: serum protein electrophoresis; UPE: urine protein electrophoresis; WM:

Waldenström macroglobulinemia.

Introduction

MG are a group of diseases that affect the bone marrow. They are caused by the clonal proliferation (one clone) of plasma cells that overproduce immunoglobulins or fragments, also called paraprotein or M-protein [1]. The released proteins serve as diagnostic markers for disease identification and quantitative biomarkers for monitoring disease progression and therapeutic response [2]. MG represents a heterogeneous and complex group of conditions with different manifestations, therapy, and prognosis. A plasma cell dyscrasia or a B-cell lymphoproliferative disorder could cause MG. Its severity varies from typically benign and asymptomatic MGUS to incurable MM and AL amyloidosis [3]. MG are a laboratory-based diagnosis that is becoming more common in older people. The primary methods include serum protein electrophoresis (SPE) and urine protein electrophoresis (UPE), which detect and quantify M-proteins in serum and urine. Immunofixation electrophoresis (IFE) is often employed to confirm the presence and type of M-protein, offering greater sensitivity than SPE alone. Serum free light chain (sFLC) assays are crucial in diagnosing, monitoring, and prognosticating MG, including MM and other plasma cell dyscrasias [4,5]. Our study aims to describe the epidemiological, biochemical, and hematological profiles of MG diagnosed in the Biochemistry department of Mohammed VI University Hospital of Oujda over more than seven years.

Material and methods

The study was a retrospective investigation conducted at Mohammed VI Teaching Hospital in Oujda. It included all patients diagnosed with SIFE positivity between Jan 1, 2016, and Sept 1, 2023. The biochemistry laboratory's immunofixation register was used for this purpose. Data collected from electronic medical records comprised age, gender, light and heavy chain isotypes, measurement of monoclonal protein, total protein, calcium, creatinine, urea, C-reactive protein (CRP), β2 microglobulin, lactate dehydrogenase (LDH), κ/λ ratio, complete blood count, blood smear, and bone marrow aspiration smear. Capillarys 2 and 3 (Sebia) and HYDRASYS 2 SCAN FOCUSING (Sebia) were used for serum protein capillary electrophoresis and immunofixation assays. In cases of light chain-only gammopathy, we screened for both IgD and IgE.

Urinary immunofixation was assessed using HYDRASYS 2 SCAN FOCUSING (Sebia) Gel electrophoresis. We evaluated the biochemical parameters with the Abbott Architect ci8200 and Alinity systems. For diagnosis, we adhered to the International Myeloma Working Group (IMWG) updated criteria for MM, SMM, and MGUS [6]. Advanced statistical analyses were conducted to compare demographic and clinical characteristics between groups. Categorical variables were compared using chi-square tests, and continuous variables were compared using ANOVA. All data were collected using Microsoft Excel, and statistical analysis was performed using SPSS Statistics Version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

314 patients were initially considered for this study because they had positive monoclonal immunoglobulin in SIFE. Of these, 90 patients were excluded since no diagnosis was attributed. Therefore, 224 patients were ultimately included in the analysis. Additionally, some patients lacked ß2 microglobulin, LDH, or M protein quantification data. While these patients were included in the overall study, they were excluded from the calculation of statistical parameters for these three variables in Table 1. There were 122 (54.46%) men and 102 (45.54%) women, for a sex ratio of 1.19. The mean age at diagnosis was 65.91 ± 11.81 years, with extremes ranging from 32 to 95 years old. Additionally, individuals aged 40 years and above constituted 89.28% of all diagnosed patients. All patients were of Moroccan nationality and were from the country's eastern region. The essential characteristics of the population diagnosed with MG are shown in Table 1. In our study, 92.85% of patients (n=208) showed a monoclonal peak. In 78.37% (n=163) of cases, the peak was in the γ globulin region, 21.15% (n=44) in the β globulin region, and 0.48% (n=1) in the α region. In terms of immunoglobulin isotypes, IgG was the most common monoclonal protein (59.82%), followed by IgA (19.64%) and IgM (6.71%). Furthermore, the only secretion of FLC was seen in 11.6% of the cases, while 2.23% exhibited biclonal gammopathy. The distribution of the monoclonal proteins found in this investigation is shown in Table 2. The distribution of the patients' diagnoses in our study was as follows: MM (78.57%), lymphoma (5.35%), plasma cell leukemia (4.02%), WM (3.57%), and MGUS (1.79%). Table 3 lists the additional clinical diagnosis, and Table 4 provides the isotype distribution for each diagnosis.For 133 individuals, urinary immunofixation was conducted, and in 95 (71.42%) of those cases, the results were positive. We conducted advanced statistical analyses to assess the differences in demographic and clinical characteristics across different types of MG. A chi-square test was performed to evaluate the association between sex and diagnosis, yielding a chi-square value of 8.024 and a p-value of 0.431, indicating no statistically significant difference in sex distribution across different diagnoses. Additionally, an ANOVA test was conducted to compare the mean age across different diagnoses, resulting in an F-statistic of 0.623 and a p-value of 0.758, suggesting no statistically significant difference in age distribution among the different MG. These findings indicate that sex and age are not significantly different across the various diagnostic categories in our study cohort.

Table 1: Principal characteristics of the 224 participants in the study.

	Mean ± SD or Median[IQR]	Minimum	Maximum	CI 95%	*n*
Age (years)	65.91±11.81	32	95	64.35-67.46	224
Male					122 (54.46%)
Female					102 (45.54%)
Total protein (*N*: 60-80 g/L)	81[33.25]	41	164	75.54-86.45	224
C-reactive protein CRP (*N*: 0-5 mg/L)	10.55[37.31]	0.13	530.49	7.21-13.80	224
Calcium (*N*: 84-102 mg/L)	91[15.25]	62	176	90.0-94.0	224
Creatinine (*N* : M : 7.2-12.5mg/L ; F : 5.7-11.1mg/L)	M 11.29 [16.56] F 8.55 [13.85]	M 3.45 F 5.01	M 252 F 231	M 7.61-14.97 F 8.00-9.46	224
Urea (*N*: 0.15-0.45 g/L)	0.41[0.605]	0.14	3.85	0.36-0.47	224
LDH (*N*: 125-243 U/L)	220.0[167.0]	52	3325	208.95-237.0	217
β2 microglobulin (*N*: 0.97-2.64 mg/L)	5.78[8.79]	0.60	90.55	4.66-6.99	136
M protein Quantification (g/L)	19.35[29.7]	2.6	100	14.95-23.90	124
κ/λ Ratio	1.94[11.46]	0.0	580	1.45-2.83	105

CI 95%: 95% confidence interval; F: female; IQR: interquartile range; M: male; SD: standard deviation, n: total count
The 95% CI represents the range within which we can be 95% confident that the true mean (for normally distributed data) or median (for non-normally distributed data) lies.

Table 2: Isotype distribution.

	N	%
IgG	**134**	**59.82**
κ	82	36.60
λ	52	23.22
IgA	**44**	**19.64**
κ	18	8.04
λ	26	11.60
IgM	**15**	**6.71**
κ	14	6.26
λ	1	0.45
FLC	**26**	**11.6**
κ	8	3.57
λ	18	8.03
Biclonal	**5**	**2.23**
Total	**224**	**100**

Table 3: The distribution of etiologies in the study's patients.

	N	%
MM	176	78.57
Lymphoma	12	5.35
Plasma Cell Leukemia	9	4.02
WM	8	3.57
MGUS	4	1.79
Chronic lymphocytic leukemia	4	1.79
Plasmocytoma	3	1.34
Unclassed	8	3.57
Total	224	100

Table 4: Isotype distribution for each diagnosis.

Diagnosis	IgG	IgA	IgM	FLC	Biclonal	*n*
MM	111	41	1	21	2	176
Lymphoma	5	0	4	2	1	12
Plasma Cell Leukemia	4	1	0	3	1	9
WM	0	0	8	0	0	8
MGUS	3	0	0	0	1	4
Chronic lymphocytic leukemia	3	0	1	0	0	4
Plasmocytoma	2	1	0	0	0	3
Unclassed	6	1	1	0	0	8
Total	134	44	15	26	5	224

Patients with MM

MM was found in 176 participants overall in our study. Of them, 79 (44.89%) were women and 97 (55.11%) were men. The mean age of the patients was 66.56 ± 11.58 years, with ages ranging from 32 to 95 years. The most frequent isotype observed in the study was IgG, accounting for 63.06% (n=111) of the cases, followed by IgA at 23.30% (n=41), FLC at 11.93% (n=21), biclonal at 1.14% (n=2), and IgM at 0.57% (n=1). We noticed 88.07% (n=155) were anemic, and 6.82% (n=12) had rouleaux development at the blood smear. The median calcium level was 90.50 mg/L [IQR: 17], ranging from 62 to 176 mg/L. 115 patients underwent urinary immunofixation; 95 instances (72.17%) had positive results.

Discussion

The study aimed to clarify the features of MG in Moroccan patients and compare our results with those of previous studies. This study's data collection was probably not exhaustive because some patient categories were not included in the analysis. The study excluded patients with incomplete medical records, those without a confirmed diagnosis, and those who did not receive follow-up care in a hospital setting. This exclusionary strategy was used to guarantee the validity and consistency of the data collected, but it might not fully represent all patients with MG.

In line with previous research [7–9], the patients in our study ranged in age from 32 to 96 years old, with a mean age of 66. This alignment supports the notion that older people are the target group of MG, as it can occur in up to 8% of the aged population [10]. In children, MG is rarely seen [11]. As the population ages and more advanced electrophoresis methods are developed, MG cases will likely increase gradually in the coming years. Moreover, our study's male predominance is consistent with the data published in the literature [12,13]. We observed that the IgG isotype predominated in our study, with IgA and IgM following suit. Comparable isotype distributions have been documented in cohorts originating from Morocco [14], Spain [15], and Tunisia [8]. This uniformity among neighboring nations highlights the regional similarity of MG patterns. Table 5 compares our dataset's monoclonal protein isotype distribution with results from other studies. Biclonal gammopathies accounted for only 2% of the cases in our study, reflecting the low prevalence reported in the literature [16]. Nonetheless, in other global investigations, the sequence of M-protein isotypes is often IgG, followed by IgM and then IgA. The potential explanation for

this variation could be the impact of ethnic differences on the expression patterns of these isotypes. Additionally, challenges in detecting IgA, particularly in minimal concentrations due to potential overlap with standard protein electrophoresis bands, especially the β1- and β2-globulins fractions, could elucidate the differences observed in comparison to the second most prevalent M-protein isotype documented in the literature.

Table 5: Comparison of our dataset's monoclonal protein isotype distribution with results from other studies.

	Our data (n=224)	Ouzzif et al [14] (n=261)	El Maataoui et al [17] (n=117)	Belouni et al [7] (n=2121)	Mseddi et al [8] (n=288)	Bergon et al [15] (n=537)	Decaux et al [12] (n=1051)	Tamimi et al [13] (n=416)
IgG	59.82	54.58	55.4	60.91	51,7	55.8	48,6	60
IgA	19.64	14.74	19.2	17.91	20,8	20.8	8,1	5.5
IgM	6.71	10.75	3.6	6.6	8,7	13.6	30,3	10
IgD	0	0	2.4	1.03	1	0.4	0	0
IgE	0	0	0	0.09	0	0	0	0
FLC (κ or λ)	11.6	0	17	10.46	13,6	6.4	3,7	24.3
Biclonal	2.23	2.79	2.4	2.82	2,1	3	9,3	0
Tricolonal	0	0	0	0.14	0	0	0	0

A significant distinction from other studies arises in the diagnosis distribution of our population, where the most prevalent diagnosis is MM rather than MGUS (Table 6). These findings contradict the existing literature but are consistent with the findings of two prior investigations conducted in Morocco [14,17]. These investigations also found a similar pattern, implying that the frequency of MM outweighing MGUS is a recurring trend in the Moroccan community. Furthermore, it is important to mention that despite our extensive research, we identified no cases of SMM among our study participants. This lack is remarkable, especially given the findings of the iStopp MM study [18], which included a large cohort of nearly 75,000 people. Their findings revealed a SMM frequency of 0.53% among people aged 40 and up. This difference in distribution between MM and MGUS in our East Moroccan study population can be attributed to a complex interplay of factors. Firstly, several limitations and potential biases inherent in this study's design and execution must be acknowledged when interpreting its findings. This investigation's retrospective nature introduces inherent limitations. Relying on preexisting data may lead to incomplete or missing information and potential selection biases. Despite efforts to mitigate these issues through rigorous data collection and inclusion criteria, the possibility of residual confounding cannot be entirely excluded. Additionally, healthcare system-related issues might have contributed to this distribution disparity. It's plausible that only symptomatic patients, more likely to exhibit abnormal SPE and SIFE results, were included in our study due to the nature of healthcare access or utilization patterns in the region. As a result, asymptomatic individuals with MGUS might have been underrepresented. These factors underscore the need for cautious interpretation of our findings and highlight the importance of considering potential biases and limitations when extrapolating results to the broader population. Future studies with larger and more diverse cohorts and efforts to address healthcare access barriers will be crucial for obtaining a comprehensive understanding of the prevalence and distribution of MG in the East Moroccan population. Other factors contributing to these findings could be demographic traits and regional variances in genetic factors, forming a different disease profile in the East Moroccan population. Furthermore, unique environmental exposures to these places may impact the development of plasma cell disorders, including MM and MGUS. Differences in diagnosis practices, criteria, and awareness can also impact the observed distribution of these diseases.

Table 6: Comparison of diagnoses in our dataset with findings from other studies.

	Our data (n=224)	Ouzzif et al [14] (n=261)	El Maataoui et al [17] (n=117)	Ouzzif et al [9] (n= 443)	Mseddi et al [8] (n=288)	Bergon et al [15] (n=537)	Decaux et al [12] (n=1051)	Tamimi et al [13] (n=416)
MM	78.57	52.77	82.1	45.65	59.26	31.3	14.1	14.6
MGUS	1.79	34.92	2.6	39.05	27.04	54.1	64.1	68
WM	3.57	3.97	1.7	5.58	4.81	2	8.7	4
Plasma Cell leukemia	4.02	0	1.7	1.86	0	0	0	0
Plasmocytoma	1.34	0.79	8.5	0.62	0	2.2	0.3	0
Lymphoma	5.35	3.97	2.6	3.51	3.12	6.3	4.2	6.5
Chronic lymphocytic leukemia	1.79	1.59	0.9	2.48	1.38	3.2	2.1	2.1

Conclusion

The MG profile reported in our group differs from established patterns in the literature, particularly in the diagnosis distribution. The distinctive features of diagnostic prevalence in our East and North Moroccan sample highlight the need for a more comprehensive knowledge of disease dynamics in this population. Further research should dive into the complexities of these disparities, elucidating the regional elements that contribute to the observed differences in disease profiles. Given that the clinical laboratory is the only setting in which all M-protein patients are followed, we believe it should play a significant role in the study of MG, serving as a focal point for comprehensive investigations and contributing to a more holistic understanding of these disorders.

Conflict of interest disclosure

The authors have no conflicts of interest to disclose.

Ethical Approval

The research studies involving human subjects strictly adhered to the ethical standards outlined in the 1964 Helsinki Declaration, as established by the relevant institutional and national research committees.

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Research Article

An approach to include the cost of consumables in biochemistry analyzer procurement on the reagent rental model alleviates hidden expenses

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Clinical biochemistry analyzers, cost per test, Reagent rental basis, Rate contract basis, Hidden costs.

Abstract

Background
Clinical biochemistry analyzers are essential for diagnosing and monitoring various diseases and conditions. However, the procurement of these analyzers is often based on the initial purchase cost, which does not reflect the total cost of ownership.

Methods
We applied a novel approach to include all hidden costs to run parameters (consumables and accessories) on a cost-per-reportable test (CPRT) basis. Fixed expenses like water purification plant, HIS connectivity, and electricity backup were assumed to be included in the cost per test itself, while the calibration cost was distributed uniformly in the calculation of CPRT itself. This CPRT was taken to compare the financial results of different bids.

Results
The cost per reportable test received after applying our novel approach in maintenance-free reagent rental basis bid was 47.4% lower than the previous cost per test for the purchased equipment.

Conclusion
This substantial decrease in cost with our novel approach reduced laboratory expenses possible with accurate comparison among analyzers with uniform specifications after eliminating the hidden expenses.

Introduction

Procuring clinical biochemistry analyzers in hospitals is a complex process, influenced by various factors, including workload, competition, and the need for a comprehensive comparison model [1]. The decision between purchasing an instrument outright or opting for a reagent rental agreement carries significant implications for laboratory requirements, budgeting and long-term needs [2,3]. This decision necessitates thoroughly evaluating several key factors, such as financial considerations, maintenance requirements, technological advancements, scalability, instrument quality and reliability and the specifics of contractual terms [4].
Some parameters to consider are listed in Table 1.

Table 1: Purchase basis and maintenance-free rental basis for laboratory equipment.

S.No.	Parameters	Purchase basis	Maintenance-free rental basis
1.	Initial investments	High	None
2.	Approval process	Complex; subject to budget	Simplified
3.	AMC/CMC	Mandatory	Not required
4.	Closed system	Possible	Guaranteed
5.	Service quality	Variable	Consistently good
6.	Reagent price	Less competitive	Competitive
7.	Condemnation process	Challenging	Not required
8.	Technology	Risk of obsolescence	Upgradable per tender terms
9.	Overall pricing	Potentially lower	Potentially higher

The best decision may be different for individual labs. The tender process may provide competition in pricing in both cases. However, in the purchase process, the focus remains concentrated on instrument pricing (and reagent purchase as per uncompetitive rate contract), while on a rental basis, cost per test is the sole focus in many cases [2]. Because of hidden costs, the concept of fair competition is easily compromised without even a sense of losing it. A calibrator set may cost around five times the reagent kit for the same parameter [5]. Also, a few underutilized tests may cost less, while some high throughput tests may be a little costlier. One may easily be cheated if factors like the number of tests, cost of calibration, cost of wash and clean solutions are not included in decision-making algorithms.

Despite the critical nature of this decision, there is a noticeable research gap in the field. Comprehensive studies considering all the hidden costs associated with both procurement strategies are lacking. Traditional models often overlook costs such as maintenance, calibration, and wash and clean solutions. Furthermore, the impact of these costs on the overall pricing strategy is often underestimated, leading to potential financial inefficiencies.

We hypothesized that a more comprehensive comparison that includes all hidden costs would provide a more accurate cost representation for justifiable comparison. Thus, a significant cost reduction may be achieved with actual competition. Therefore, this study was planned with our novel calculative approach to test the above hypothesis.

Methodology

The study/tender process was conducted at the Dr Ram Manohar Lohia Institute of Medical Sciences in Lucknow. The tender was published online in accordance with government guidelines. The duration of the contract was set to be 60 months.

The bid was divided into two components: Technical and Financial.

1. **Technical bid:** A comprehensive package was planned, which included all necessary items such as a water plant, computer, printer, and HIS connectivity. Preferably, a minimum of 2 or 3 bids must be accommodated per the state's tender guidelines to ensure competitive pricing. Both dry and wet chemistry-based bids were permitted, with a disclaimer that certain items not necessary as per technology (like a water plant for dry chemistry platforms) were exempted.

2. **Financial bid:** This component involved a price comparison for 30 commonly used biochemical parameters. The monthly tests were calculated by averaging the throughput of the last six months, as taken from the Hospital Information System (HIS). Similarly, the cost of accessories/consumables was also calculated by dividing the cost per pack by the number of tests that could be run with the pack. The Cost per Test (CPT) was determined as follows:

$$\text{Cost per test (CPT)} = \frac{\text{Cost of reagent kit}}{\text{Test per kit}}$$

Cost per test calculation for parameters

Table 2 presents the cost per test (CPT) calculation for 30 parameters. The CPT was calculated using the formula CPT = c/T, where c is the kit cost and T is the number of tests.

Table 2: Cost analysis of diagnostic kits for various tests.

S.No.	*Parameters	Cost of kit [c]	No of tests (T)	CPT (=c/T)
1	Albumin			
…	…	…	…	…
30	Uric Acid			

* All parameters' details in Table 4.

Other unit costs

The cost of accessories/consumables to run each parameter was also calculated as described for CPT calculation and added as a separate column for each (Annexure for CPT, Table 4).

Calibration requirement data

The calibration requirement data was directly obtained from our instruments and was found to be 0.97 per 100 tests. It was averaged to be once per hundred tests in general. The ultimate aim was to include the cost of calibration in decision-making. Caution was taken to ensure that the cost per test remained primary while other costs were added (in total/fraction). It was not individualized for each test, as the same calibrator set could be utilized for many tests.

Cost of calibration per test (CPCT)

The cost of calibration per test (CPCT) was calculated using the following formula: $CPCT = r \times [(V_c \times n) + V_d]/100$

Where:

- r is the rate per µL, calculated as the cost of the calibrator set divided by the total volume of the calibrator set,
- Vc is the volume required to calibrate once,
- n is the number of times the calibrator has to be run (if in duplicate, n=2; in triplicate, n=3),
- Vd is the dead volume (the volume that can't be picked up by the sample probe from a cuvette).

All these data were taken on the company's letterhead and verified from kit/calibrator inserts (submitted in PDF along with the tender document as a mandatory condition).

Cost per reportable test (CPRT):

It is the total cost of the reagent to run an individual parameter. It includes CPT, cost per accessories/ consumables, and CPCT.

S.No.	Parameters*	No. of test per month(n)	Name of the calibrator	Cost of Calibrator	Vol of calibrator per set(µL)	Rate/µL (r)	Calibrator vol to be used per cycle of calibration(in µL)(a)	Times of calibration run (in (numerical)-(b)	Dead volume- (c)	Calibrator vol per calibration [d=(aXb)+c]	Cost per calibration CPC (e = r X d)	Cost of calibration per test CPCT [=e/100] **
1	Albumin											
..	…….											
30	Uric Acid											

*All parameters' details in Table 4. ** Taking one calibration per 100 tests (as per interpretation from previous six-month data from our lab). The parameter sequence should be the same as mentioned in the bid (col no 1-30 remains the same as in 'Format for Submitting the Financial Bid.

Final financial bid

The final financial bid charges included an annual maintenance contract (AMC), comprehensive maintenance contract (CMC), consumables, uninterruptible power supplies (UPS), water purification system, battery, and HIS connectivity. The maintenance charge (AMC/CMC, consumables like halogen lamps) cost of any other requirements like water purification system, UPS, batteries, and hospital information system (HIS) connectivity for data transfer was also assumed to be zero. The vendor was supposed to include it in the pricing of reagents/ calibrators. Anything not mentioned in the final financial bid chart was considered a hidden charge and assumed to be zero as an essential tender condition. The cost of controls was intentionally not included in the tender as third-party controls are desirable for better quality per the national accreditation board for testing and calibration laboratories (NABL) norms.

The cost per test is the key factor among initial verification/ validation, calibration, QC run, repeat run, and dilution. Since we cannot predict it exactly, it would be better to accept it as essential maintenance/ expanse to manage hassle-free services. If fixing it is the vendor's responsibility, it would be natural for him to keep the cost per test on the higher side to avoid losses.

Cost per reportable test (CPRT; column 9) was multiplied by the test volume (column 3) of the same parameter and put in column 10 as total cost (predicted per month on given test volume.

The sum of all rows in column 10 (total offered value of the above parameters) was compared among different vendors to decide the lowest bidder. Any breakdown was fixed to be charged with a daily fine unless the backup was not provided to ensure smooth running without affecting turnaround time (TAT), which is often considered a key marker of the lab's efficiency. The condition of providing upgradation in terms of technology and throughput

on the departmental committee's demand was implemented to address any such need during the contract period.

Formulas used for final financial bid calculation

Table 3 presents the formulas used for the final financial bid calculation for 31 parameters. The Cost per Run Test (CPRT) was calculated using the formula CPRT = CPT + cost of accessories to run each parameter + Cost of consumables + Others + CPCT, where CPT is the cost per test and CPCT is the cost of calibration per test (Annexure for CPRT, Table 4).

Table 3: Cost Analysis of Diagnostic Tests.

1	2	3	4	5	6	7	8	9	10
S.No.	**Parameter**	**No. of test per month (n)**	**Cost per test (CPT)**	**Cost of accessories to run each parameter**	**Cost of consumables**	**Others**	**Cost of calibration per test (CPCT)**	**CPRT**	**Total cost (=nXCPRT)**
1	Albumin	2000							
..	…….	…….	…….	…….	…….	…….	…….	…….	…….
30	Uric Acid	1800							
Total offered value									

Results

The comparison of the cost per test (CPT) rates supplied by the same company (rate contract basis for the purchased equipment) with the reagent rental basis tender procedure resulted in a significant reduction in reagent pricing for the given 30 parameters. The overall reduction in cost was found to be 47.4%.

Table 4: Cost analysis and reduction in reagent pricing.

S.N.	Parameters	No. of test per month (n)	CPT (Old) (%)	CPRT (New) (%)
1	Albumin	2000	100	78
2	ALP	5000	100	34
3	ALT	5000	100	63
4	Amylase	800	100	21
5	AST	5000	100	60
6	Calcium	200	100	93
7	Cholesterol	1500	100	91
8	CK	300	100	90
9	CKMB	100	100	9
10	Creatinine Jaffe	7000	100	100
11	Direct Bilirubin	5000	100	94
12	Ferritin	200	100	9
13	GGT	400	100	71
14	Glucose HK	5000	100	77
15	HDL	1500	100	68
16	hs-CRP	100	100	86
17	Iron	450	100	92
18	ISE (Na, K, Cl)	5000	100	39
19	LDH	100	100	87
20	LDL	1500	100	7

21	Lipase	700	100	100
22	Magnesium	400	100	90
23	Phosphorous	500	100	92
24	Total Bilirubin	5000	100	45
25	Transferrin	100	100	49
26	Triglyceride	1500	100	54
27	Total Protein	1500	100	96
28	UIBC	300	100	88
29	Urea	6500	100	81
30	Uric Acid	1800	100	90

Reduction (%)	52.6
Net reduction (%)	**47.4**

ALP: Alkaline phosphatase, ALT: Alanine transaminase, AST: Aspartate transaminase, CK: Creatinine kinase: CK-MB: Creatine kinase-MB, GGT: gamma-glutamyl transferase, HDL: High density lipoprotein, hs-CRP: High sensitive creactive protein, ISE: Ion selective electrode, LDH: Lactate dehydrogenase, LDL: Low density lipoprotein, UIBC: Unsaturated iron binding capacity.

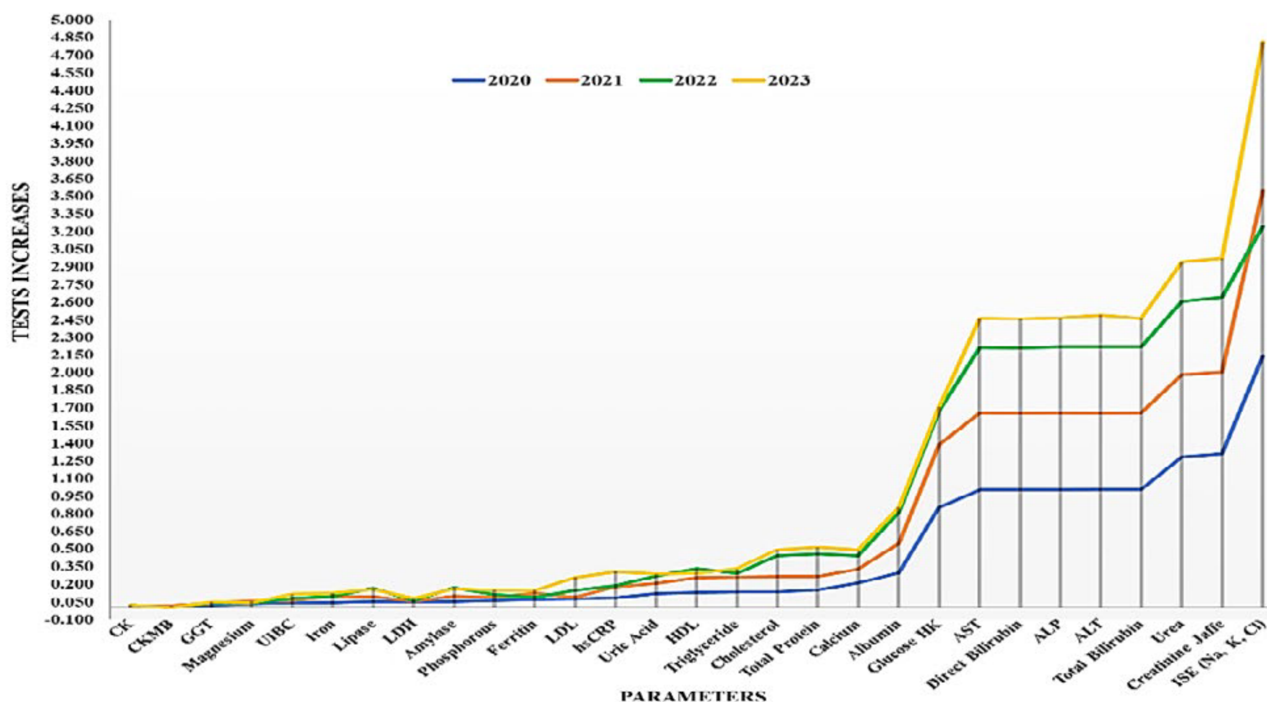
Parameter-wise cost reduction

A detailed analysis of each parameter revealed varying degrees of cost reduction. For instance, the cost of albumin testing was reduced by 22%, while the reduction for amylase was as high as 79%.

Total cost reduction

As there was no manufacturer certified/ provable way available to calculated CPRT for older prices. We have compared cost per reportable test (CPRT) for post tender prices to cost per test (CPT) of older prices. The cost per test with old prices (CPT, old) was higher than the cost per repeatable test with new prices (CPRT, new) for all parameters except lipase, which was equal after negotiation. Thus, CPT (old) has been taken as 100% and CPRT (new) has been represented as a percentage fraction of CPT (old). The data has been represented as percentages to avoid revealing the exact figures. When considering the total cost for all parameters, the new CPRT resulted in a net reduction of 47.4% in cost compared to the old CPT. This substantial decrease in cost demonstrates the effectiveness of the reagent rental basis tender procedure in reducing laboratory expenses.

Figure 1: Year-wise test load versus parameters.



On analysis of the last 4 years annual workload, we found that the mean (standard deviation) increase in the number of tests per consecutive year was 166 % (SD 24%), 126% (SD 34%), and 116% (SD 24%) in years 2021, 2022 and 2023 respectively.

Discussion

The study aimed to develop a novel approach for procuring clinical biochemistry analyzers that considered all hidden costs, including maintenance. The hypothesis was that this approach would provide a more accurate representation of the actual cost of clinical biochemistry analyzers, leading to more cost-effective decisions and improved hospital budgeting. The study compared the cost per test (CPT) rates supplied by the same company (rate contract basis for the purchased equipment) with the reagent rental basis tender procedure. This substantial decrease (47.4%) in cost demonstrates the effectiveness of the novel approach in reducing laboratory expenses and supports the hypothesis of the study. High workload, fair competition, precise calculations and inclusion of sufficient bidders may be attributed to the same. The maximum cost saving was in ISE electrolytes, as electrodes were quoted as free by the lowest bidder in our case. However, it was not the same with other participant bidders. Although the cost reduction per individual parameter was maximum for CKMB, ferritin and LDL, the overall cost saving was minimal due to a much lesser test volume of these parameters. This can be interpreted as a strategy of the bidder to provide low test volume kits on throw away prices to get the contract with minimum quoted price. The LDL is done by calculation at many centres (not at our institute), the leadership of the vendor company might have misunderstood it this way and hence underquoted for the same. However, the intrest of the institute was not compromised (rather amplified) as we invited the bid at the real workload. Though the reagent contracts may vary widely between countries, the spirit of organizing a fair competition shall always remain. Elimination of hidden costs is one such way to provide an equal platform. We have excluded the taxes while calculating as it may vary per state-specific regulations. The study also provides a detailed analysis of the cost reduction for each parameter, which can help identify the most cost-efficient parameters and optimize the laboratory workflow. The previous studies have focused on the cost of reagents and calibrators alone, without considering the other costs that are involved in running the analyzers. Smith et al. (2018) compared the cost of reagents and calibrators for different types of analyzers but did not include the cost of maintenance, labor, electricity, or waste disposal [6]. Similarly, a study by Jones et al. (2020) compared the cost of reagents and calibrators for different brands of analyzers but did not account for the cost of accessories, consumables, or calibration [7]. These studies may have underestimated the cost of clinical biochemistry analyzers and led to biased or inaccurate decisions. Furthermore, the previous studies have used a rate contract basis for the purchased equipment, which is a conventional approach involving the purchase of the analyzers, reagents, and calibrators separately. This approach places the burden of maintaining the analyzers and providing the accessories and consumables on the users. This approach also leads to the overconsumption of reagents and calibrators and underutilization of the analyzers; Lee et al. (2018) used a rate contract basis for the purchased equipment and found that the cost of reagents and calibrators was higher than the cost of the analyzers [8]. Similarly, a study by Chen et al. (2017) used a rate contract basis for the purchased equipment and found that the utilization rate of the analyzers was lower than the optimal level [9]. During the contract period, we assumed that the workload increase would be random and proportional. To validate this, we analyzed the pattern of workload increase over the past four years. The workload increase was consistent for most parameters, as shown by the low standard deviation each year. However, there were notable exceptions for specific parameters, specifically CKMB, CK-total, and GGT. These findings lend support to our initial hypothesis. It's crucial to highlight that all parameters saw a proportionally more significant increase between 2020 and 2021. This was mainly due to the decrease in workload during the COVID-19 pandemic. Through multiple discussion sessions with multiple vendors, we ensured that our specifications were at par with at least four of them; to the best of our understanding, all four were approved by the United States Food and Drugs Administration (USFDA). Only three participated, while the one with dry chemistry-based technology refrained, possibly due to pricing issues. The study has some limitations that should be acknowledged. First, the study used a convenience sample size from one laboratory, which may limit the generalizability of the results to other laboratories. Thus, each lab must quote the true data (tests per month) in its bid. Any mismatch may severely compromise the purpose; only the tests done in the lab must be included. A slight deviation in a high throughput test may create a significant difference. If some new or low yield parameter is to be included, its workload should be taken as 100 (minimum size of kit on most of the biochemistry analyzers) so that it can be finished before expiry (preferably at least 6 months in our case). Smaller labs may prefer cost-effectiveness, but if a lab has a sufficient workload, procuring an approved autoanalyzer may be necessary to restrict low-quality/technology bidders. Secondly, the study only considered the cost of reagents and calibrators, which may not capture the total cost of running the clinical biochemistry analyzers. Future studies should include other costs, such as human resources, electricity, and waste disposal, to assess the total cost of ownership of the clinical biochemistry analyzers. However, human resources recruitment is complicated due to expertise and state regulations. We have practiced at least two staff training sessions in our biochemistry lab for a long time in compliance with NABH (National Accreditation Board of Hospital and Health Care Providers) guidelines for good laboratory practices. The vendors also comply as staff training may indirectly affect business outcomes. Though we did not include staff training as an essential condition in our procurement process, it would be better to include it for better compliance. However, we must clearly define training specifications like staff strength, number of sessions, standardization like inviting specific external professional expert(s), different expanses and the outcome expectations must be clearly defined to ensure the quality as well as helping the vendors in planning the budget. As our proposed model is maintenance-free reagent rental

basis installation, this cost for the same may be included either in reagent charges or as a separate entity in the final financial bid. With the later approach, payments may be cleared after the expected outcomes are achieved satisfactorily.

Conclusion

This study introduces a comprehensive method for procuring clinical biochemistry analyzers that considers all hidden costs, including maintenance, consumables, accessories, and calibration. By incorporating these costs into the Cost Per Reportable Test (CPRT), we observed a significant reduction of 47.4% in costs when using a maintenance-free reagent rental basis bid, compared to the previous cost per test for purchased equipment. This approach allows for a more accurate comparison among analyzers with similar specifications by effectively accounting for hidden expenses. As a result, this method can potentially revolutionize the procurement process of clinical biochemistry analyzers, leading to more cost-effective diagnostic services. Future research should focus on validating this approach across diverse laboratory settings to confirm its effectiveness and applicability. Furthermore, future studies should consider including additional factors such as human resources, staff training, and other operational expenses to provide a more holistic view of the total cost of ownership.

Declaration

This original article has not been published before and is not currently being considered for publication elsewhere. All authors have read and approved the study. The authors declare no conflict of interest.

Ethical Statement

This study does not involve any human or animal samples. It is a cost comparison study conducted using machinery.

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Research Article

Unveiling the Role of Magnesium: Insights into Insulin Resistance and Glycemic Control in Type 2 Diabetes

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Keywords

Hypomagnesemia, T2DM, Glycemic Control, HOMA-IR, Glucose Metabolism

Abstract

Background

Diabetes mellitus (DM) is a significant and escalating global health concern, with Type 2 DM (T2DM) constituting approximately 90% of all DM cases. Magnesium (Mg) plays a crucial role in various physiological processes. Hypomagnesemia is prevalent in T2DM patients. The severity of hypomagnesemia correlates with glycemic control and is linked to the development of complications associated with T2DM.

Aim

The objective of our study was to evaluate the occurrence of hypomagnesemia in patients with T2DM and explore its association with both glycemic control and the development of complications in rural and urban populations.

Methods

The study consisted of 300 diabetic and 100 non-diabetic patients between 31 and 55 years of age. Fasting blood glucose, post-prandial blood glucose, and magnesium levels were estimated using a fully automated analyzer, Selectra Pro-XL. HbA1c was measured using Bio-Rad D10. Insulin levels were calculated using the chemiluminescence method. HOMA-IR was also assessed using a formula: fasting insulin (U/mL) multiplied by fasting plasma glucose (FPG) (mmol/L) divided by 22.5.

Result

Magnesium levels were significantly lower in diabetic patients (1.34±0.29) than in the control (2.17±1.87) with $p<0.0001$. FBS (267.67±89.78 mg/dL vs. 167.87±76.87 mg/dL, $p<0.0001$), PPBS (376.87±112.87 mg/dL vs. 287.90±99.98 mg/dL, $p<0.0001$), HbA1c (9.54±2.6 % vs. 7.23±1.8 %, $p<0.0001$), Insulin (17.21±8.98 IU/mL vs. 14.87±5.98 IU/mL, $p=0.039$) and HOMA-IR (7.32±3.67 vs. 6.13±0.99, $p=0.012$) were significantly elevated in the hypomagnesemia group than the normal magnesium levels. Magnesium levels were negatively correlated with FBS ($r=-0.465$; $p<0.0001$), PPBS ($r=-0.596$; $p<0.0001$, HbA1c ($r=-0.765$; $p<0.0001$), Insulin ($r=-0.454$; $p<0.0001$), and HOMA-IR ($r=-0.325$; $p<0.0001$).

Conclusion
Our study suggests that monitoring serum magnesium levels is crucial for individuals with Type 2 diabetes mellitus (T2DM) to manage hypomagnesemia, mitigate associated complications, and optimize overall care.

Introduction

Diabetes mellitus (DM) is a major public health problem all over the world, particularly in developing nations. DM is classified as a chronic medical condition [1,2]. According to the International Diabetes Federation, approximately 415 million people aged 20-79 are affected by diabetes [2,3]. In 2015, the global prevalence of diabetes mellitus (DM) was significant, with projections suggesting an increase of approximately 200 million cases by 2040. DM is a chronic metabolic disorder characterized by sustained hyperglycemia, primarily stemming from impaired insulin secretion, insulin resistance, or a combination of both [4,5]. Type 1 DM (T1DM) results from an absolute deficiency in insulin secretion, whereas Type 2 DM (T2DM) is primarily attributed to relative insulin deficiency and its resistance [5,6]. Type 2 diabetes mellitus (T2DM) constitutes approximately 90% of all diagnosed cases, and it is particularly concerning that the Indian population tends to develop it at a younger age than Western populations, often with minimal weight gain [7]. This highlights the complex interplay of genetic, environmental, and lifestyle factors contributing to the onset of T2DM in different populations [8]. Mg is the fourth most abundant cation in the human body and is necessary for various physiological mechanisms. These mechanisms include the phosphorylation of glucose during glucose metabolism, DNA synthesis, and other elementary biological processes [9,10]. Mg plays an important role in these mechanisms. Furthermore, magnesium is believed to play a significant role in the metabolic process of insulin-mediated cellular glucose uptake and in regulating insulin effects [11]. Hypomagnesemia can be a complication of diabetes itself, and there is evidence to suggest that consuming an adequate amount of magnesium may lower the chance of developing T2DM [10,11]. Magnesium is essential in selecting physiological activities, including glucose metabolism and insulin sensitivity [11]. In addition to its role in insulin resistance, magnesium is involved in insulin secretion and glucose metabolism. Individuals who have diabetes or who are at risk of developing the condition may benefit from maintaining adequate magnesium levels by diet or supplementation [12]. This may help reduce insulin resistance and improve glycemic control. Several problems associated with diabetes, including insulin resistance, glucose intolerance, and dyslipidemia, have been linked to hypomagnesemia. Patients with type 2 diabetes frequently experience hypomagnesemia, the severity of which is related to both glycemic control and complications [12]. Individuals with type 2 diabetes are at a higher risk of encountering renal impairment, experience quicker disease progression, and achieve unfavorable outcomes.

Waheed et al., 2022, concluded that the consumption of alcohol in patients suffering from diabetes mellitus is more likely to develop hypomagnesemia than in diabetic patients who do not use alcohol ($p<0.0001$). The study found that the frequency of alcohol intake was also closely associated with the development of Hypomagnesemia ($p=0.002$) [15]. Mutations in genes involved in magnesium transport, dietary habits, and diarrhea caused by diabetic autonomic neuropathy or metformin can impair magnesium absorption in the intestine. It is critical to learn more about blood magnesium levels in Type 2 DM (T2DM) patients and how they relate to glycemic control and complications in DM because hypomagnesemia is associated with DM. As a result, an in-depth investigation of these areas requires additional research. Therefore, we have initiated a study investigating these health issues, specifically focusing on rural and urban communities. This study aimed to examine the magnesium levels and their correlation with HbA1c, FBS, and PP blood sugar to assess glycemic control in patients with Type 2 Diabetes Mellitus (T2DM).

Material and Method

Patient Recruitment

The study was conducted in the Department of Medicine in collaboration with the Department of Biochemistry, Uttar Pradesh University of Medical Sciences, Saifai, Uttar Pradesh. This study includes 300 diabetic patients who were not on medication (magnesium supplements and metformin drugs) who visited the outpatient department in the Department of Medicine, and 100 age-sex-matched controls who did not have diabetes or other metabolic diseases were recruited from the same department.

Sample Collection

4 mL of venous blood sample was collected in plain, fluoride, and EDTA vials of both subjects via vein puncture in the morning after 12 hours of fasting. EDTA sample was used to estimate HbA1c, and a plain sample was used to estimate magnesium levels. Post-prandial blood sample was also collected after 2 hours of meal in fluoride vial.

Inclusion Criteria

This study recruited 300 diagnosed T2DM outpatients based on the 1999 WHO criteria. Before enrolment, subjects were asked to sign an informed consent form.

Exclusion Criteria

Patients who had diabetes combined with acute complications such as ketoacidosis and hyperosmolar coma, acute or chronic inflammation, severe hepatic and renal dysfunction, malignant tumors, and other endocrine and metabolic disorders.

Estimation of Blood parameters

The magnesium level was measured using the photometric method on the fully automated analyzer Selectra Pro-XL (EliTech).

Fasting and post-prandial glucose levels were estimated using the GOD-POD method on the same instrument. HbA1c was measured using high-performance liquid chromatography on a Bio-Rad D-10 analyzer. Serum insulin levels were calculated using immuno-chemiluminescence on the fully automated analyzer Abbott Architect 1000SR.

Calculation of HOMA-IR

To measure insulin resistance, a modified homeostasis model assessment of insulin resistance (HOMA—IR) was used. This model is defined as fasting insulin (U/mL) multiplied by fasting plasma glucose (FPG) (mmol/L) divided by 22.5 [19].

Data Analysis

Data was analyzed by using SPSS version 24 software, Chicago, USA. The data was represented by mean and standard deviation. Pearson correlation was used to see the correlation between two variables. The student t-test and chi-square test were used to see the significant differences between variables. P-value <0.05 was considered as statistically significant.

Result

Table 1 reveals the demographic characteristics of the study population. No significant differences were found between age and gender, showing an adequate match for both groups. 228 (76%) diabetic patients showed low magnesium levels in their serum, while only 14 (14%) of normal subjects had low magnesium levels, which was found to be significant.

As evident in Table 2, fasting and post-prandial glucose levels were significantly higher in diabetic patients than in control. Insulin level and insulin resistance were also elevated in diabetic patients than the control with p<0.0001, respectively. The magnesium level was significantly reduced in diabetic patients (1.34±0.29 mg/dL) than the control (2.17±1.87 md/dL) with p<0.0001.

Table 1: Distribution of age, gender, and serum magnesium in the study population.

Variables	Diabetic (N=300) N (%)	Non-diabetic (N=100) N (%)	p-value
Age (years)			0.139
31-40	133(44)	55(55)	
41-50	101(34)	30(30)	
>50	66(22)	15(15)	
Gender			0.512
Male	184(61)	65(65)	
Female	116(39)	35(35)	
Magnesium levels			**<0.0001***
Low (<1.7 mg/dL)	228(76)	14(14)	
Normal (> 1.7 mg/dL)	72(24)	86(86)	

The chi-square test was used to compare the group. *p<0.05 was considered as statistically significant.

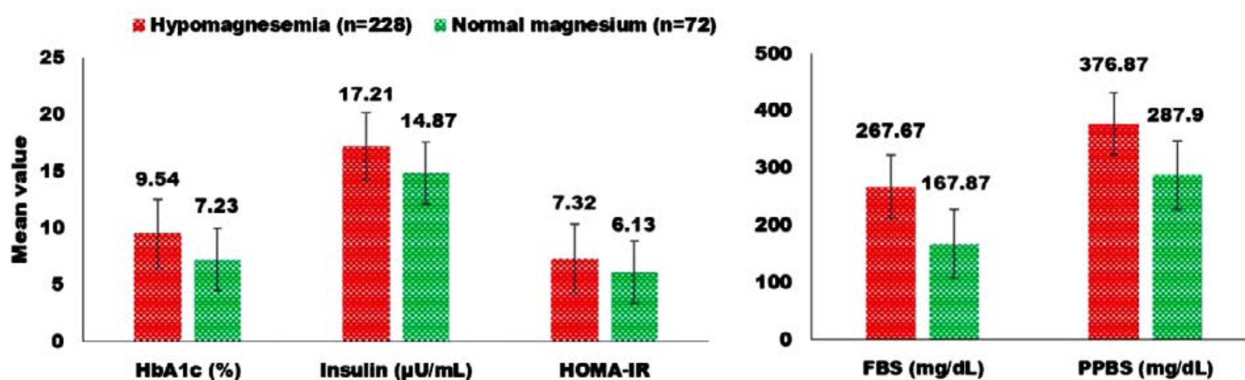
Table 2: Comparison of biochemical parameters in diabetic and non-diabetic subjects.

Variables	Diabetic (N=300) Mean±SD	Non-diabetic(N=100) Mean±SD	p-value
FBS (mg/dL)	175.87±98.98	88.98±10.98	<0.0001*
PPBS (mg/dL)	245.98±123.09	110.87±19.98	<0.0001*
HbA1c (%)	9.87±3.87	5.78±1.87	<0.0001*
Insulin (IU/mL)	18.98±12.87	10.98±8.67	<0.0001*
HOMA-IR	6.97±6.32	2.65±2.13	<0.0001*
Magnesium (mg/dL)	1.34±0.29	2.17±1.87	<0.0001*

FBS: Fasting Blood Sugar, PPBS: Post-prandial Blood Sugar, HbA1c: Glycosylated hemoglobulin, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. *p<0.05 was considered as statistically significant.

Figure 1 represents the status of diabetic markers based on hypo and normal magnesium levels. FBS (267.67±89.78 mg/dL vs. 167.87±76.87 mg/dL, p<0.0001), PPBS (376.87±112.87 mg/dL vs. 287.90±99.98 mg/dL, p<0.0001), HbA1c (9.54±2.6% vs. 7.23±1.8%, p<0.0001), Insulin (17.21±8.98 IU/mL vs. 14.87±5.98 IU/mL, p=0.039) and HOMA-IR (7.32±3.67 vs. 6.13±0.99, p=0.012) were significantly elevated in the hypomagnesemia group than the group with normal magnesium levels.

Figure 1: Comparison of diabetic parameters in low and normal magnesium levels.



FBS: Blood Sugar, PPBS: Post-prandial Blood Sugar, HbA1c: Glycosylated hemoglobulin, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. *p<0.05 was considered as statistically significant.

Magnesium levels were negatively correlated with FBS (r=-0.465; p<0.0001), PPBS (r=-0.596; p<0.0001, HbA1c (r=-0.765; p<0.0001), Insulin (r=-0.454; p<0.0001), and HOMA-IR (r=-0.325; p<0.0001) (Table 4).

Table 3: Correlation of diabetic parameter with serum magnesium levels.

Variables	Magnesium levels	
	r-value	p-value
FBS (mg/dL)	r=-0.465	p<0.0001*
PPBS (mg/dL)	r=-0.596	p<0.0001*
HbA1c (%)	r=-0.765	p<0.0001*
Insulin (IU/mL)	r=-0.454	p<0.0001*
HOMA-IR	r=-0.325	p<0.0001*

r: Pearson Correlation, FBS: Fasting Blood Sugar, PPBS: Post-prandial Blood Sugar, HbA1c: Glycosylated hemoglobulin, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. *p<0.05 was considered as statistically significant.

Discussion

Our research revealed that the age group ranging from 31 to 55 years old comprised the biggest proportion of patients diagnosed with Type 2 Diabetes Mellitus (T2DM). Furthermore, there were a greater number of male patients than there were female patients. We found that the majority of patients with type 2 diabetes (T2DM) had hypomagnesemia, which is characterized by low serum magnesium concentrations. On the other hand, a smaller proportion of patients (24%) had normal serum magnesium levels. Hence, it would appear that hypomagnesemia is the most common condition among people who have Type 2 Diabetes Mellitus (T2DM). This suggests that the risk of hypomagnesemia is the same for people of all ages who have type 2 diabetes. Nevertheless, it is worth noting that we found a statistically significant inverse association between serum magnesium levels and both fasting and postprandial blood sugar levels (PPBS) among the patients (p< 0.0001). Our research indicates that there is a connection between the degree of hypomagnesemia that patients with type 2 diabetes mellitus (T2DM) experience and the degree to which they can control their blood sugar

levels. Specifically, we found a statistically significant negative association between the levels of magnesium in the serum and the value of HbA1c ($p < 0.0001$). The findings of this study indicate that as serum magnesium levels decline, HbA1c levels tend to increase, which suggests a lack of glycemic control. The extent of hypomagnesemia in type 2 diabetes is indeed connected with glycemic control, as our data demonstrates, which lends weight to the assumption that this correlation exists. Hypomagnesemia, a deficiency of magnesium in the blood, can exacerbate complications in individuals with diabetes due to its crucial role in glucose metabolism and insulin action. In diabetes, hypomagnesemia is often seen alongside poor glycemic control, insulin resistance, and diabetic complications like neuropathy and cardiovascular disease. Magnesium deficiency further hampers insulin sensitivity which impairs glucose uptake by cells and exacerbates inflammation and oxidative stress, all of which are central to the pathogenesis of diabetes complications. Moreover, hypomagnesemia is associated with dyslipidemia and hypertension, compounding the cardiovascular risk in diabetic patients. Thus, addressing hypomagnesemia alongside glycemic control and other metabolic factors becomes essential in mitigating the risk and severity of complications in diabetes. Our study observed that out of 300 patients, 228 had hypomagnesemia with increased fasting glucose and HbA1c values. This indicates that magnesium deficiency can impair insulin action, leading to elevated fasting blood glucose levels and poor glycemic control reflected in higher HbA1c levels over time. High HOMA-IR was also increased in hypomagnesemia patients, indicates that magnesium deficiency through supplementation or dietary adjustments may help improve insulin sensitivity and lower HOMA-IR levels. This approach potentially mitigates the risk of diabetes and its associated complications. Another study was conducted by Hashim et al., 2023, parallel to our research; they found that the prevalence of hypomagnesemia was 21.5%. They also observed the significant age difference, duration of T2DM, and diabetic complications, as well as all laboratory parameters except high-density lipoprotein cholesterol between the hypomagnesemia and normal magnesium level groups. Glycated hemoglobin (HbA1c), fasting plasma glucose, and duration of diabetes independently predicted serum Mg levels in T2DM patients. Finally, they concluded that serum Mg was used as an indirect biomarker of glycemic control in T2DM patients, whereby hypomagnesemia indicates poor control [14]. Furthermore, serum magnesium levels may be influenced by complications of diabetes mellitus (DM). Lecube et al. noted that serum magnesium concentrations were significantly reduced in Type 2 DM (T2DM) patients compared to non-diabetic subjects. They also found a significant negative correlation between serum magnesium concentration, fasting blood sugar (FBS) levels, and HbA1c [16]. These findings are also in concordance with our study results. Our findings also suggested the negative correlation between magnesium with fasting glucose, HbA1c, insulin, and HOMA-IR with $p<0.05$. Another study was also parallel to this finding, study conducted by Doddigarla et al., 2016, observed that inverse Pearson correlation coefficient, r (−0.376), (−0.689), (−0.05), (−0.05), (−0.40), (−0.14), (−0.342) and (−0.548) were established when HbA1c of control and T2DM patients were compared with control and T2DM patients of serum Cr, Zn, Mg and SOD variables in that order.

In addition, another study conducted by El-said et al., 2015 observed serum Mg levels were significantly reduced in type 2 diabetic patients compared to the control group with mean ±SD (1.29 ± 0.31 mg/dl) versus (2.41 ± 0.13 mg/dl) with p value < 0.001. There were highly significant negative correlations between serum Mg levels and HbA1c, fasting glucose, and insulin resistance with (r = -0.969, -0.894, -0.653) respectively, p-value < 0.001. Their finding suggested that hypomagnesemia is closely linked to type 2 diabetes mellitus, and it is strongly correlated to glycemic control. They also recommend measuring serum Mg in type 2 diabetes, and patients who need supplementation should be considered [17]. Polat et al., 2024, also observed plasma and erythrocyte Mg^{++} levels in people with diabetes were significantly lower than in the control group plasma and erythrocyte Mg^{++} levels. They suggested in study that magnesium deficiency was associated with high HbA1c and high glucose levels [18]. Erinc et al., 2015 observed a strong negative correlation between serum magnesium levels and HbA1c (r=-0.316, p<0.001). There was also a weak negative relationship between Mg and serum fasting glucose, insulin, and HOMA-IR (r=-0.167 p=0.004, r=-0.167 p=0.003, and r=-0.198 p=0.001, respectively) [19]. In addition, Yossef et al. reported a statistically significant negative correlation between serum magnesium levels and fasting blood sugar (FBS), postprandial blood sugar (PPBS), and HbA1c [20]. This finding provides additional evidence for the correlation between serum magnesium levels and indicators related to glycemic management, which is consistent with the results of our investigation. Pillay et al. enrolled 744 patients; most patients were female (527; 70.8%) and were diagnosed with Type 2 diabetes (T2DM) (633; 85.1%) with a mean age of 52.3 (SD 15.6 years). The prevalence of hypomagnesemia was found to be 8.44%. Hypomagnesemia was associated with poor glycemic control (r=–0.16, p<0.0001). A significant relationship was observed between glycemic control and hypomagnesemia in males (r=–0.21, p=0.0038) but not in females (r=–0.011, p = 0.81). No significant relationship was evident between hypomagnesemia and renal dysfunction (r =–0.064, p=0.11). Finally, they concluded that hypomagnesemia in patients with DM was associated with poorer glycemic control in the male population, potentially increasing the risk of adverse health outcomes [21]. Although there are some similarities between our study and previous findings, other variations could explain the observed inconsistencies. The disparities may arise due to discrepancies in research conditions, environmental influences, food patterns, or lifestyle choices within the studied groups. Our study encountered several limitations. First, we could only conduct the study with a relatively small sample size due to time constraints. Second, the study was carried out at a single center, which may limit the generalizability of our findings. Third,

the possibility of hospital bias cannot be disregarded, as the study was conducted in a tertiary care hospital setting. Another important consideration is that magnesium predominantly exists as an intracellular cation. This raises questions about the validity of using serum magnesium concentrations as a proxy for assessing the impact of magnesium on different physiological states.

Conclusion

Low serum magnesium concentrations are commonly observed in patients with Type 2 diabetes mellitus (T2DM). The extent of hypomagnesemia in T2DM correlates with the degree of glycemic control achieved. Given magnesium's essential role in various physiological processes and it's potential to induce complications in individuals with T2DM, monitoring serum magnesium concentrations should be prioritized for all T2DM patients. Effective management strategies should be implemented to address any deficiencies detected, thus helping to mitigate the risk of associated complications and optimize patient outcomes.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

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Research Article

Comparative Analysis of HbA1c Estimation Using Immunoturbidimetry and High-Pressure Liquid Chromatography Methods in Non-Dialysis Chronic Kidney Disease Patients

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Keywords

Chronic Kidney Disease, HbA1c, Turbidimetric Inhibition Immunoassay, High-Pressure Liquid Chromatography, Carbamylated Hemoglobin.

Abstract

Background

Chronic kidney disease (CKD) concomitant with diabetes mellitus (DM), anemia and uremia. Thus, monitoring HbA1c levels presents a complex clinical challenge.

Methods

This analytical cross-sectional study was conducted from May 2022 to April 2023 at Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. We compared HbA1c values obtained by the turbidimetric inhibition immunoassay (TINIA) and high-pressure liquid chromatography (HPLC) methods among non-dialysis CKD patients (n=127).

Results

HbA1c was not detectable among 27 patients by TINIA but measurable with HPLC, all being's anemic. The remaining 100 patients, it was detectable by both the methods. Among these 100 patients, linear regression analysis showed a very strong positive correlation between TINIA-HbA1c and HPLC-HbA1c (R2=0.861; p<0.0001). The agreement between methods was substantial (Cohen's kappa 0.657; p<0.0001). However, HbA1c levels were detected significantly higher with HPLC (Median 7.9, IQR 2.7) than that of TINIA (Median 7.0, IQR 2.9;p=0.025) in diabetics while the difference was not significant in non-diabetic group with both HPLC (Median 5.4,IQR 0.8) and TINIA (Median 5.1,IQR 1.1). Carbamylated Hb (CHb; as detected by HPLC as a side product) was correlated to both HbA1c by HPLC (r=0.299;p=0.007) and TINIA (r=0.336;p=0.006) as well as to serum urea levels (r=0.439;p<0.0001).

Conclusion

HPLC estimates all HbA1c patients in our study group while TINIA failed to do so in around 21.26% cases. The very low hemoglobin levels and high carbamylated hemoglobin were apparent as two most common causes. Also, the values with TINIA are significantly lower in comparison to HPLC among diabetics with CKD.

Introduction

Chronic kidney disease (CKD) is a significant health concern, impacting approximately 27% of the population, with a heightened occurrence among individuals with diabetes [1,2]. Glycosylated hemoglobin (HbA1c) serves as a crucial biomarker for managing diabetes mellitus (DM), as it reflects long-term glucose control. Accurate HbA1c measurements are vital for reducing vascular complications by maintaining glycemic control [1]. However, stringent HbA1c targets may pose risks [2], and the measurement methods—chromatographic or immunochemical measurement methods can yield different results [3]. In CKD patients, carbamylated hemoglobin (CHb), produced when hemoglobin reacts with urea-derived isocyanate [4,5], can interfere with HbA1c readings [6,7]. Despite advancements in analytical methods, elevated CHb levels in CKD patients continue to challenge the accuracy of HbA1c measurements, complicating the assessment of glycemic control in patients with uremia. Addressing this issue necessitates a nuanced understanding of the biochemical interactions between CHb and HbA1c, as well as the implementation of mitigation strategies to ensure reliable HbA1c assessments in this patient population. To measure HbA1c, various analytical techniques have been employed, with chromatography being the most prevalent technique because of its effectiveness in detecting total glycated hemoglobin [8]. The National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have been instrumental in enhancing the precision of HbA1c measurement [9,10]. Despite advancements in quality control, variability still exists among the methods certified by the NGSP for HbA1c testing. Additionally, one can employ various laboratory techniques to determine HbA1c levels in the blood. Studies have consistently shown notable discrepancies between these analytical methods. Biological variation sets the permissible total error for HbA1c at 3.0%, while NGSP standards allow up to 6.0% [11,12]. Based on the above facts, this study aims to compare the efficacy of the immunoturbidimetric inhibition immunoassay (TINIA) with that of high-pressure liquid chromatography (HPLC) in the analysis of HbA1c. In addition, there is no correlation between CHb, iron, and creatine levels.

Methodology

Study design and participants

This analytical cross-sectional study was conducted at the Department of Biochemistry in collaboration with the Department of Nephrology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India. HbA1c values obtained by HPLC and TINIA were compared in CKD patients [HbA1c-D (n = 100) and HbA1c-ND (n = 27)]. These patients were confirmed to have CKD by a nephrologist and divided into two groups: those with diabetes mellitus (DM) (n = 40) and those without DM (n = 60) (Table 1). The severity of the disease was divided by the KIDGO guidelines. The repetition was performed with fresh calibration and controls to validate the results. Exclusion criteria included patients undergoing routine hemodialysis or peritoneal dialysis, renal transplant recipients, those unwilling to participate, or individuals under 18 years of age. The Institutional Ethics Committee approved the study (reference number: IEC-48/22), and informed consent was obtained from all participants in accordance with the principles of the Declaration of Helsinki [13] and institutional ethical guidelines.

Table 1: On the basis of detectable and not-detectable HbA1c by TINIA method, status of demographical, biochemical, and hematological variables in chronic kidney disease (CKD) patients.

Variables	Detectable (n=100) N(%)	Not-detectable (n=27) N(%)	p-value
Gender			
Male	63(63.0)	16(59.3)	0.115
Female	37(37.0)	11(40.7)	
Anemia			
Yes	82(82.0)	27(100.0)	0.013*
No	18(18.0)	0	
HTN			
Yes	63(63.0)	08(29.6)	0.002*
No	37(37.0)	19(70.4)	
Severity			
Stage 3	25(25.0)	8(29.6)	0.023*
Stage 4	37(37.0)	16(59.3)	
Stage 5	38(38.0)	03(11.1)	

	Median (IQR)	**Median (IQR)**	
Urea (mg/dL)	81.0 (64.68)	157.5(63.2)	<0.0001*
Creatinine (mg/dL)	3.4 (3.55)	7.9(6.7)	<0.0001*
Iron (µg/dL) Male Female	48.0(22.0) 50.1(21.8) 48.0(16.8)	24.0(12.9) 23.9(11.5) 15.0(12.2)	<0.0001* <0.0001* <0.0001*
HPLC- HbA1c (%)	5.9 (1.9)	5.7(1.4)	0.040*
TINIA- HbA1c (%)	5.6 (1.6)	-	-
CHb	1.8 (0.7)	2.5(1.8)	<0.0001*
CHb/Hb ratio	0.18 (0.10)	0.30 (0.22)	<0.0001*
eGFR	23.8 (16.1)	25.4(18.5)	0.165
UACR	8.2 (45.5)	7.4(5.7)	0.232
Hb(g/dL)	10.4 (3.3)	6.6(0.8)	<0.0001*
RBC Count (million/mm3)	3.8(1.0)	3.4(1.0)	0.500
MCV (fL)	89.2(8.0)	85.3(11.0)	0.208
MCH (pg)	28.4(3.0)	27.6(4.0)	0.424
MCHC (g/dL)	31.8(1.0)	32.3(2.0)	0.582
RDW (%)	14.0(2.0)	12.0(1.0)	0.493
PCV (%)	34.5(8.0)	30.6(12.0)	0.596

HbA1c: glycated hemoglobin, DM: Diabetes mellitus, NDM: No diabetes mellitus. HTN: Hypertension. IQR: Interquartile range, HPLC-Hba1c: High pressure liquid chromatography- Glycated hemoglobin, TINIA: Turbidimetric inhibition immunoassay, CHb: Carbamylated hemoglobin, eGFR: Estimated glomerular filtration rate, UACR: Urine albumin creatinine ratio, Hb: Hemoglobin, RBC: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red blood cell distribution width, PCV: Packed cell volume. The Man-whitney test was used to calculate the p-value. * p-value <0.05 was considered as statistically significant.

Sample collection

Venous blood (2 mL) was collected from each vial, plain and EDTA, after information regarding age, sex, comorbidities, and patient consent was obtained. The samples were then centrifuged at 3000 rpm for 10 minutes to separate plasma and serum for biochemical estimation. Blood samples collected for the study were stored at -20 °C for six months (free only once) [14].

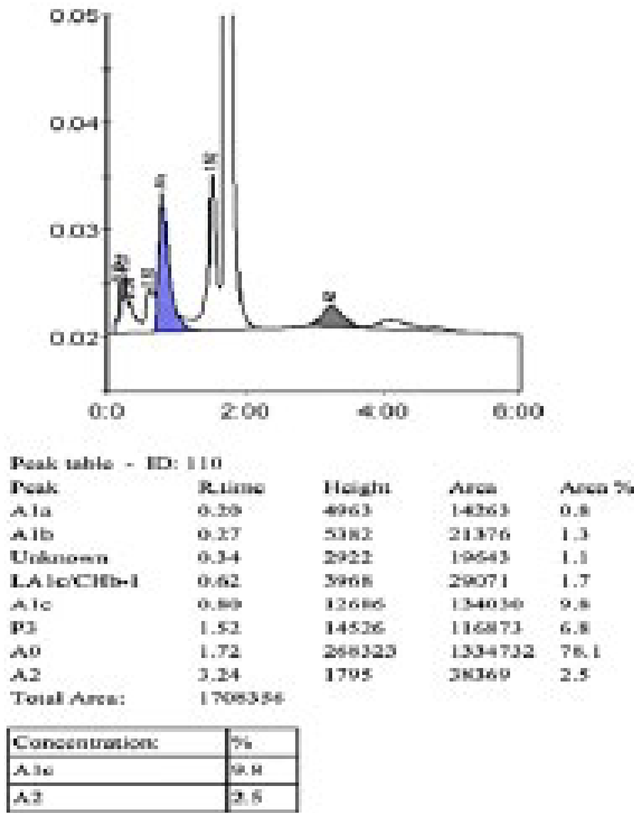
Biochemical estimation

Urea and creatinine levels were analyzed using commercial reagents on a fully automated analyzer (Cobas 6000; Roche Diagnostics).

HbA1c and carbamylated Hb estimation using the HPLC technique

HbA1c levels were measured using ion-exchange HPLC (Bio-Rad D10 Laboratories, Inc., Hercules, California, USA). The three-minute short program (HbA1c mode) was primarily utilized for HbA1c quantification. Carbamylated hemoglobin is represented as the area percentage (Figure 1.).

Figure 1: Representative chromatogram for HbA1c and CHb on HPLC.



Estimation of HbA1c using the TINIA technique

HbA1c was estimated using the immunoturbidimetry technique using commercial reagents on a fully automated integrated analyzer (Cobas 6000, Roche Diagnostics). This method involves analyzing HbA1c levels without needing to measure total hemoglobin levels. The absorbance of HbA1c bound to the particles was measured and found to be proportional to the percentage of HbA1c in the samples.

Data analysis

The baseline characteristics of the participants are summarized as numbers, percentages, and medians (IQR). The chi-square and Mann-Whitney test were used for group comparisons. Pearson's correlation coefficients were used to assess the correlations between urea, creatinine, HbA1c, CHb, and hemoglobin levels. The two methods (HPLC-HbA1c and TINIA-HbA1c) were compared using Bland-Altman plots. Scatter plots of the test data and reference methods were created, and their linear relationship was calculated using a linear regression model (OLR may be applied when the correlation coefficient exceeds 0.9 or 0.99 (slope (b) and y-intercept (a)). All analysis was performed using SPSS software version 24 (Chicago, IL, USA). Statistical significance was set at $p<0.05$.

Results

Glycated hemoglobin (HbA1c) detectability and its association with renal markers in CKD patients

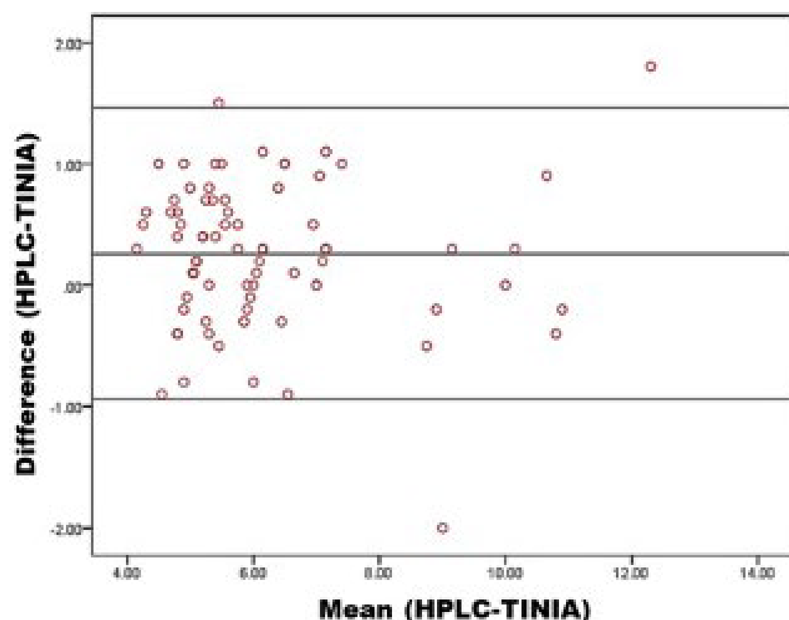
The gender distribution showed a slight male predominance in both HbA1c detectable (HbA1c-D) and non-detectable (HbA1c-ND) groups ($p=0.115$). Anemia and hypertension were significantly more prevalent in the HbA1c-D group, with 82% ($p=0.013$) and 63% ($p=0.002$) of the subjects affected, respectively. CKD severity was also significantly higher in the HbA1c-D group ($p=0.023$). Biochemical analysis revealed significantly lower urea levels in the HbA1c-D group (81.0 mg/dL, IQR: 64.68) compared to the HbA1c-ND group (157.5 mg/dL, IQR: 63.2, $p<0.0001$). Creatinine levels also showed a similar pattern, with lower levels in the HbA1c-D group (3.4 mg/dL, IQR: 3.5) than in the HbA1c-ND group (7.9 mg/dL, IQR: 6.7, $p<0.0001$). Conversely, iron levels were significantly higher in the HbA1c-D group (48.0 µg/dL, IQR: 22.0) compared to the HbA1c-ND group (24.0 µg/dL, IQR: 12.9, $p<0.0001$). HPLC-HbA1c levels were significantly higher in the HbA1c-D group (5.9%, IQR: 1.9) than in the HbA1c-ND group (5.7%, IQR: 1.4, $p=0.040$). Hb levels were also significantly higher in the HbA1c-D group (10.4, IQR:3.3) than in the HbA1c-ND group (6.6, IQR: 0.8, $p<0.0001$). CHb and CHb/Hb ratios were significantly lower in the HbA1c-D group (1.8, IQR:0.7; 0.18, IQR:0.1) compared to the HbA1c-ND group (2.5, IQR:1.8; 0.3,

IQR:0.2, p<0.0001, respectively). No significant differences were observed in UACR, eGFR, RBC count, MCV, MCH, MCHC, RDW, and PCV (p> 0.05) (Table 1).

Correlation and agreement of HbA1c levels measured using HPLC and TINIA methods

The Bland-Altman method was used to calculate the mean difference (bias) between the two techniques. The plot from this analysis indicates agreement between the two methodologies. As shown in Figure 2, 95% of the values were within the range of the mean ± 2 standard deviations (SD) around the study mean. The data revealed no significant differences in HbA1c values measured using HPLC and TINIA methods (Figure 2).

Figure 2: Bland Altman plot for HPLC-HbA1c- and TINIA- HbA1c method.



Further, patients were classified into two categories: those with HbA1c levels between 5.7% and 6.4%, indicating an increased risk of developing diabetes, and those with HbA1c levels of ≥6.5, considered confirmed diabetic. Both methods demonstrated substantial agreement with Cohen's kappa (κ) values of 0.657 (p<0.0001) (Table 2).

Table 2: Concordance of HbA1c between two methods HPLC and IT.

HPLC-HbA1c	IT-HbA1c			Cohen's kappa (κ)	p-value
		≥6.5%	≥6.5%	0.657	<0.0001*
	≥6.5%	65	2		
	≥6.5%	12	21		

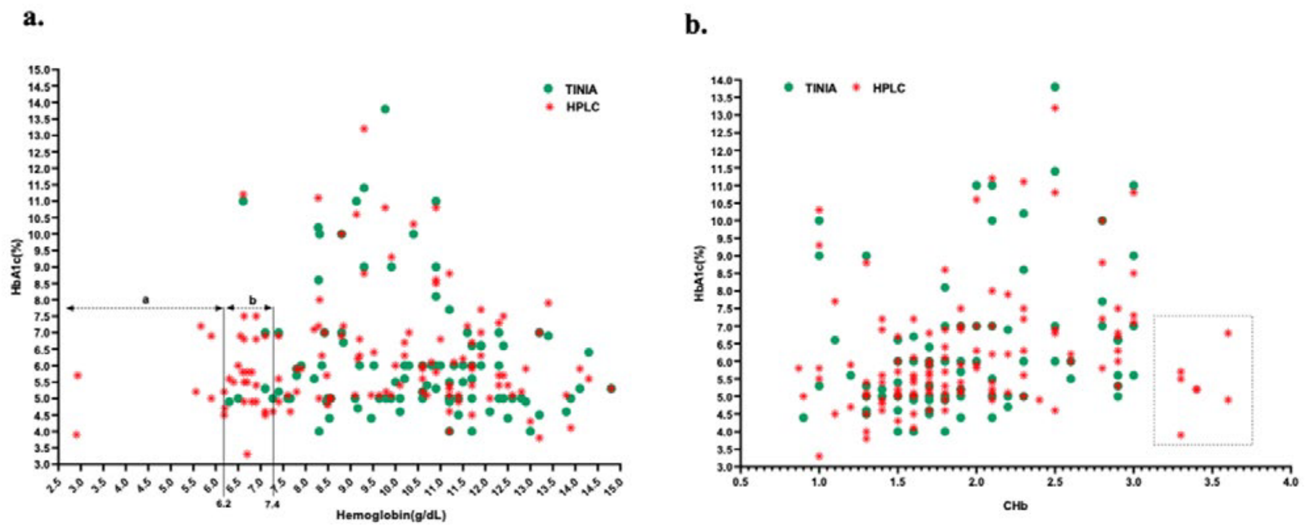
HbA1C; glycated hemoglobin; HPLC, high-performance liquid chromatography; IT, immunoturbidimetry. *p-value <0.05 was considered as statistically significant.

TINIA detection limit compromise with Hb and CHb levels in CKD patients

Figure 3a illustrates that in patients with CKD who had hemoglobin (Hb) levels below 6.2 g/dL, the TINIA method failed to detect HbA1c in 22.2% of cases. Conversely, for patients with Hb levels ranging from 6.2 to 7.4 g/dL, TINIA was able to detect HbA1c in 29.2% of cases, and detection was successful for all patients with Hb levels above 7.4 g/dL.

In contrast, the HPLC method successfully detected HbA1c in all CKD cases. Particularly, with an increased level of CHb above 3.0, TINIA failed to detect HbA1c. Similarly, the ratio of CHb to Hb was higher in patients where TINIA detection failed (Figure 3b). Interestingly, TINIA-HbA1c and HPLC-HbA1c have a very strong positive correlation (r=0.928, p<0.0001). Also, there was a significant concordance, with 87.4% of the variance in the TINIA result explained by HPLC results (R2=0.861, p<0.0001) (Figure 4d). A positive correlation was observed between CHb and HPLC-HbA1c (r=0.299, p=0.003) and TINIA-HbA1c (r=0.336, p=0.002) (Figure 4a and 4b). The urea level also demonstrated a positive correlation with CHb (r=0.439, p<0.0001) (Figure 4c). CHb and creatinine also showed a weak positive correlation (r=0.30, p=0.003). Hb and creatinine showed a strong negative correlation (r =-0.492, p<0.0001) (Table 3).

Figure 3: Scattered plot represents the status of HbA1c versus Hemoglobin and b. carbamylated hemoglobin (CHb).



Doted box (a) represents the cases with <6.2 g/dL Hb; Horizontal line (b) donated the cases had Hb: 6.2 to 7.4 g/dL and square box denoted the cases with CHb >3.0 detected by HPLC method.

Table 3: Pearson correlation among the biochemical parameters

Variables	CHb	Hb (g/dL)	TINIA-HbA1c (%)	HPLC-HbA1c (%)	Creatinine (mg/dL)
CHb	1	r=-0.383 p<0.0001*	r=0.336 p=0.002*	r=0.300 p=0.003*	r=0.222 p=0.027*
Hb (g/dL)		1	r=0.063 p=0.579	r=0.051 p=0.625	r=-0.492 p<0.0001*
TINIA-HbA1c (%)			1	r=0.928 p<0.0001*	r=-0.081 p=0.472
HPLC-HbA1c (%)				1	r=-0.096 p=0.340
Creatinine (mg/dL)					1

HbA1c: glycated hemoglobin; HPLC: High-pressure liquid chromatography, TINIA: Turbidimetry inhibition immunoassay, Hb: Hemoglobin, CHb: Carbamylated hemoglobin. *p-value <0.05 was considered as statistically significant.

Clinical and laboratory parameters analysis in diabetic versus non-diabetic CKD patients with detectable HbA1c levels Anemia was observed in 82.5% of the diabetic group (p=0.758) and hypertension in 72.5% (p=0.108), with no significant difference in CKD severity (p=0.846). Urea levels averaged 87.3 mg/dL (IQR: 71.9) in diabetics and 79.4 mg/dL (IQR: 58.9) in non-diabetics (p=0.207). Creatinine was similar for both groups, with diabetics at 3.3 mg/dL (IQR: 2.7) and non-diabetics at 3.4 mg/dL (IQR: 3.8) (p=0.332). Iron levels were higher in non-diabetics, averaging 47.0 µg/dL (IQR: 31.7) versus 33.7 µg/dL (IQR: 19.5) in diabetics (p=0.042). HPLC-HbA1c levels were lower in non-diabetics at 7.0% (IQR: 2.9) compared to 7.8% (IQR: 2.7) in diabetics (p=0.001). Similarly, HbA1c-TINIA levels were lower in non-diabetics at 5.0% (IQR:1.05) versus 6.7% (IQR: 2.2) in diabetics (p<0.0001). CHb levels were also lower in non-diabetics at 1.7 (IQR:0.5) compared to 2.3 (IQR: 1.2) in diabetics (p=0.018). The eGFR was higher in non-diabetics at 22.0 (IQR: 18.6) versus 10.9 (IQR: 7.8) in diabetics (p=0.029). Other parameters, including CHb/Hb ratio, UACR, Hb, RBC count, MCV, MCH, MCHC, RDW, and PCV, showed no significant differences (p> 0.05) (Table 4).

Figure 4: Pearson correlation graph: a. Carbamylated Hemoglobin vs. HbA1c-HPLC, b. Carbamylated Hemoglobin vs. HbA1c-TINIA, c. Carbamylated Hemoglobin vs. Urea, d. A linear regression scattered plot by comparing using two different methods: HbA1c-HPLC and HbA1c-TINIA.

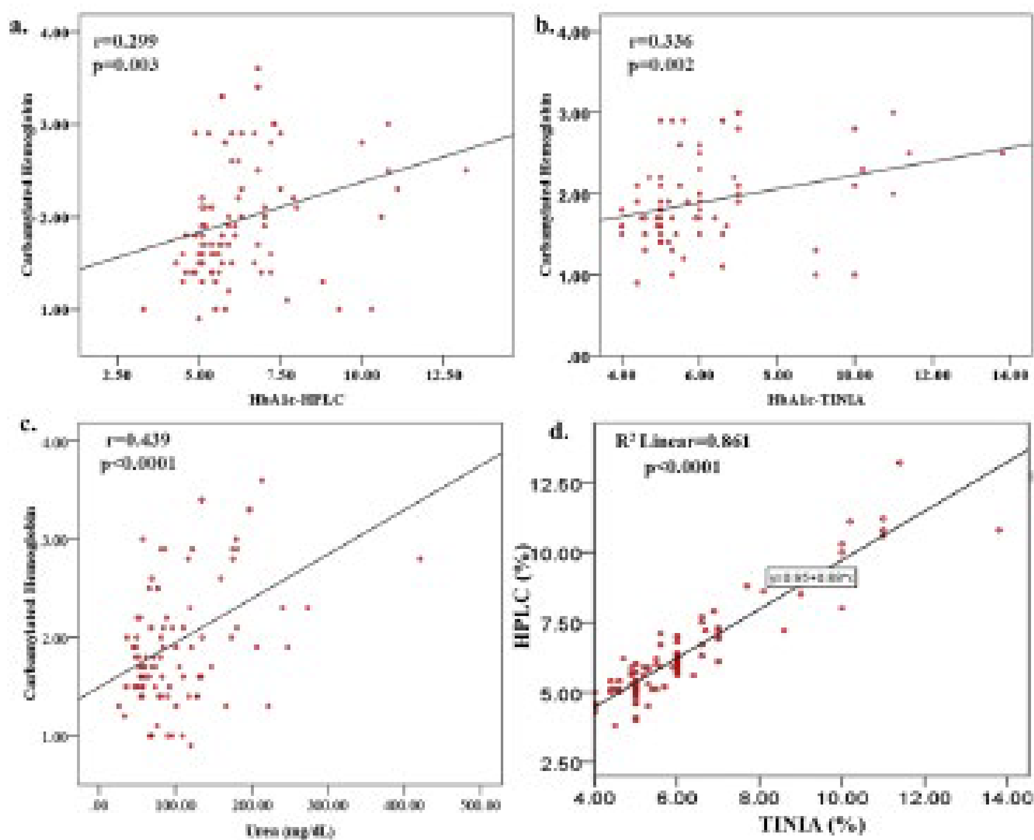


Table 4: On the basis of diabetic and non-diabetic in detectable HbA1c by TINIA method, status of demographical, biochemical, and hematological variables in chronic kidney disease (CKD) patients.

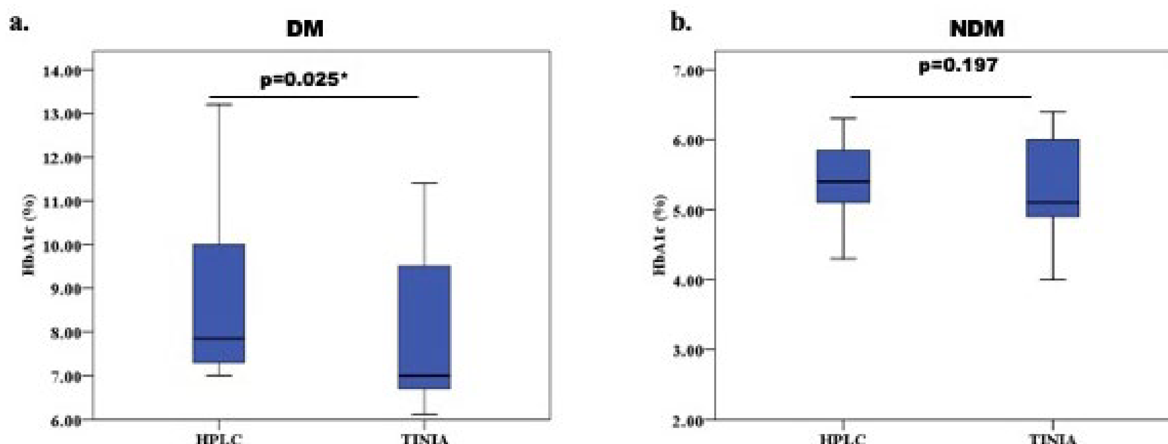
Variables	DM (n=40)	NDM (n=60)	p-value
Gender Male Female	 30(75.0) 10(25.0)	 33(55.0) 27(45.0)	0.025*
Anemia Yes No	 33(82.5) 07(17.5)	 49(81.7) 11(18.3)	0.758
HTN Yes No	 29(72.5) 11(27.5)	 34(56.7) 26(43.3)	0.108
Severity Stage 3 Stage 4 Stage 5	 25(25.0) 37(37.0) 38(38.0)	 8(29.6) 16(59.3) 03(11.1)	0.846
	Median (IQR)	**Median (IQR)**	
Urea (mg/dL)	87.3(71.9)	79.4(58.9)	0.207
Creatinine (mg/dL)	3.3(2.7)	3.4(3.8)	0.332
Iron (µg/dL) Male Female	33.7(19.5) 56.5(19.7) 53.0(47.7)	47.0(31.7) 49.7(26.7) 45.0(12.7)	0.042* 0.048* 0.034*
HPLC- HbA1c (%)	7.8 (2.7)	7.0 (2.9)	0.001*
TINIA- HbA1c (%)	6.7 (0.1)	5.0 (1.1)	<0.0001*
CHb	2.3(1.2)	1.7(0.5)	0.018*
CHb/Hb ratio	0.19 (0.1)	0.16(0.1)	0.512
eGFR	10.9(7.8)	22.0(18.6)	0.029*
UACR	31.0(13.4)	29.3(14.5)	0.056
Hb(g/dL)	8.54(1.4)	9.55(1.3)	0.128
RBC Count (million/mm3)	3.4(1.1)	3.3(1.0)	0.541
MCV (fL)	88.1(9.0)	89.9(9.0)	0.801
MCH (pg)	28.4(2.0)	27.9(3.0)	0.984
MCHC (g/dL)	32.0(2.0)	31.7(1.0)	0.528
RDW (%)	14.9(1.0)	14.5(1.0)	0.773
PCV (%)	31.4(9.0)	30.4(8.0)	0.972

HbA1c: glycated hemoglobin, DM: Diabetes mellitus, NDM: No diabetes mellitus. HTN: Hypertension. IQR: Interquartile range, HPLC-Hba1c: High pressure liquid chromatography- Glycated hemoglobin, TINIA: Turbidimetric inhibition immunoassay, CHb: Carbamylated hemoglobin, eGFR: Estimated glomerular filtration rate, UACR: Urine albumin creatinine ratio, Hb: Hemoglobin, RBC: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red blood cell distribution width, PCV: Packed cell volume. The Man-whitney test was used to calculate the p-value. * p-value <0.05 was considered as statistically significant.

Comparison of HbA1clevels estimated in diabetic and non-diabetic patients by HPLC and TINIA methods

The box-and-whisker plot compares the HbA1c levels estimated using HPLC and TINIA in diabetic and non-diabetic patients. In DM group exhibited; HPLC-HbA1c%was (median: 7.9, IQR: 2.7) significantly higher than TINIA-HbA1c% (median: 7.0, IQR: 2.9, p=0.025), while in NDM group the HPLC-HbA1c and TINIA-HbA1c were not found significant (Figure 5. a, b).

Figure 5: Comparison of HbA1c by two different methods: HPLC and TINIA. a. Among DM, b. Among NDM.



DM: Diabetes mellitus, NDM: No diabetes mellitus, HPLC: High pressure liquid chromatography, TINIA: Turbidimetric inhibition immunoassay

Discussion

HbA1c has been a preferred tool for diabetes monitoring, but its accurate estimation in CKD patients remains a challenge. With anemia, iron deficiency, and uremia common among these patients, the selection of an appropriate method for HbA1c estimation is essential. While HPLC is preferred method worldwide, alternative methods such as the immunoturbidimetry (TINIA in our study) are gaining attention for their cost-effectiveness and operational simplicity, making them a promising solution for resource-limited settings as it does not require a separate instrument in contrast to HPLC. Studies have shown that the correlation between the immunoturbidimetric method and HPLC suggests that the former is a reliable substitute for HbA1c measurement in diabetic patients. The literature's comparison of both techniques in the CKD group is limited, especially given its complexity [15-19]. The present study aimed to evaluate the agreement and efficacy between HPLC and TINIA methods in CKD patients as well as the correlation between CHb, urea, Hb, and HbA1c levels. The present study revealed that TINIA-HbA1c and HPLC-HbA1c have a very strong positive correlation ($r=0.928$; $p<0.0001$) signifying 87.4% concordance among the two techniques. The Bland-Altman analysis indicates a strong concordance between the HPLC and TINIA methods for measuring HbA1c levels. This agreement suggests that either method can be used interchangeably without compromising accuracy. The categorization of patients based on HbA1c levels, and the substantial agreement indicated by Cohen's kappa values ($\kappa=0.657$; $p<0.001$) offers a strong foundation for stratifying patients according to diabetes risk, which is crucial for glycemic monitoring and treatment. Our findings, which are in line with the advocacy of Genc S et al. [20], compare the HbA1c values obtained by TINIA and HPLC to assess the concordance between these methods. Their results showed that the mean HbA1c values were 7.789% (± 2.106%) for TINIA and 7.797% (± 2.552%) for HPLC. However, on further comparison, the two methods did not show significant differences in the non-diabetic group, suggesting that both methods are equally suitable for estimating HbA1c levels in non-diabetic individuals. Conversely, within the diabetic CKD cohort, HPLC significantly overestimates HbA1c among diabetics (median; 7.8) in comparison to TINIA (median 6.7).

Most importantly, HbA1c estimation was not possible with TINIA in 27 patients (21.25%), all these patients were severely anemic (Hb<7.4 g/dl). However, HPLC provided value to all the patients. This may be owing to the dependence of the TINIA method on hemoglobin estimation. The manufacturer's insert mentions that Hb<4 g/dl can't be determined. In our study, we found that TINIA failed to calculate HbA1c in 100% of cases with Hb below 6.2. This highlights that TINIA should not be preferred among severely anemic patients. This limitation is less pronounced but still present (29.2%) in patients with Hb levels between 6.2-7.4 g/dL, indicating the presence of some additional interfering factor such as carbamylated Hb, and iron deficiency.

Our findings demonstrated that hemoglobin and iron levels are significantly higher in non-diabetic patients with chronic kidney disease (CKD) as compared to diabetic CKD patients. This observation aligns with other studies highlighting the prevalence of anemia in CKD patients [21,22]. According to a meta-analysis, iron deficiency did not affect the HbA1c levels [23]. In addition, patients with iron deficiency anemia have been found to have a higher glycation rate, which may be due to the higher malondialdehyde levels, a lipid peroxidation metabolite, observed in this population, thus enhancing Hb glycation [24,25]. Furthermore, anemia affects hemoglobin metabolism, thereby impacting HbA1c levels [26]. In our study 85.8% (n=109 out of total 127) of the CKD patients were anemic. The HPLC method gives different peaks for the HbA1c and CHb, but the literature suggests that CHb levels interfere with HbA1c estimation in HPLC as well as TINIA. In our study, we observed that in all the patients with CHb >3.25 by HPLC, TINIA failed to detect

the HbA1c levels. CHb was positively correlated with HPLC-HbA1c (r=0.299; p=0.003), TINIA-HbA1c (r=0.336;p=0.002) and urea (r=0.439;p<0.0001). The reaction of Hb with urea-derived isocyanate forms CHb, which may spuriously cause high values of HbA1c by interfering with the estimation method [5]. Thus, precise assessment of glycemic control in patients with uremia remains problematic. In CKD patients, urea is often dissociated into isocyanate in vivo, reacts with hemoglobin, and forms CHb in a process called carbamylation [27]. However, studies have shown that high blood urea levels interfere with the estimation method, leading to spuriously high HbA1c values [28]. The CHb formation may represent a possible interference in HPLC during HbA1c measurement because the chemical modification at the N-terminal valine results in both molecules co-eluting almost simultaneously, subsequently producing an overlapping peak [29]. Naresh et al. (2018) studied 60 patients and divided them into three groups: acute kidney injury (AKI), CKD, and controls. CHb was highest in CKD patients, intermediate in AKI patients, and lowest in normal patients [30] and it was concluded that CHb can be used to differentiate between AKI and CKD patients. They also reported that carbamylated hemoglobin levels are more directly related to urea than creatinine levels [30]. Wynckel et al. conducted a similar study and stated that the longer the duration of exposure of proteins to high urea concentrations, the higher the amount of CHb formed [31]. According to Stim et al., the relationship between CHb and blood urea nitrogen was linear, but in the case of renal failure patients, it was exponential [32]. Sabrinathan et al. 2020 estimated HbA1c levels in 50 patients with diabetes by comparing the same methods and showed a good positive correlation (r =0.992) [33]. Conversely, the strong negative correlation between Hb and creatinine (r=-0.492) highlights an inverse relationship, affirming lower erythropoiesis in renal failure. Although we did not measure other interfering factors like vitamin (B12, B9, C and E) levels or drug history (aspirin, dapsone, sulfasalazine) levels, the failure of TINIA in HbA1c calculation in 21.25% of cases clearly depicts the superiority of HPLC. Both techniques show minimal interference with CHb. Further studies comparing upcoming methods for HbA1c estimation, like capillary electrophoresis (CE) with HPLC, may be performed by fixing the above limitations in the study design.

Conclusion

This study provides valuable insight into glycemic monitoring by comparing HPLC and TINIA for HbA1c estimation. HPLC detects the HbA1c peak directly, while TINIA is dependent on the biochemical estimation of Hb. TINIA fails to estimate HbA1c in both anemia and high CHb levels (owing to uremia), which are integral to CKD. Thus, HPLC should be preferred among CKD for HbA1c estimation. Alternatively, non-Hb-based tests, such as GA (glycated albumin) or serum fructosamine, may be used.

Declaration

This original article has not been previously published and is not currently being considered for publication elsewhere. All authors read and approved the study.

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Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

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Commentary

Cancer biomarker concentration changes during tumor progression

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Keywords

serum biomarker; cancer biomarker changes; biomarker upregulation; biomarker downregulation; biomarker diagnostics

Abstract

Introduction
Most circulating cancer and other disease biomarker concentrations increase during disease progression, roughly correlating with tumor burden or disease severity. During the biomarker discovery phase, several studies (some published in high-impact journals) report decreases in serum biomarkers at the time of disease diagnosis or during progression (in comparison to control, non-diseased populations). It is suggested these biomarker decreases between normal and diseased populations may have utility in diagnostics.

Methods
We briefly examine if a serum cancer biomarker concentration is likely to decrease as cancer progresses through empirical data.

Results
We propose a simple model, which, if correct, would suggest that in most cases, the biomarker decrease during disease progression could be an artifact or epiphenomenon (thus representing false discovery). Our suggestion is supported by the very few examples of decline of serum biomarkers during cancer development and progression.

Conclusions
The notion that a serum biomarker concentration could be inversely associated with tumor burden seems to be an epiphenomenon.

Introduction

Cancer biomarkers have important clinical applications, including screening, diagnosis, prognosis, and monitoring of patients' therapeutic response. Many contemporary discovery technologies, including genomics, proteomics, metabolomics, and other omics, revolutionized the way we identify and validate new biomarkers. New, and potentially clinically useful biomarkers are still published frequently in the literature. For example, a few candidate serum biomarkers for gliomas have just been published in the journal Science Advances by combining genomics and spatial multidimensional proteomics [1]. Despite the unequivocal progress and technological refinements in discovering new biomarkers, very few, if any, new serological markers have

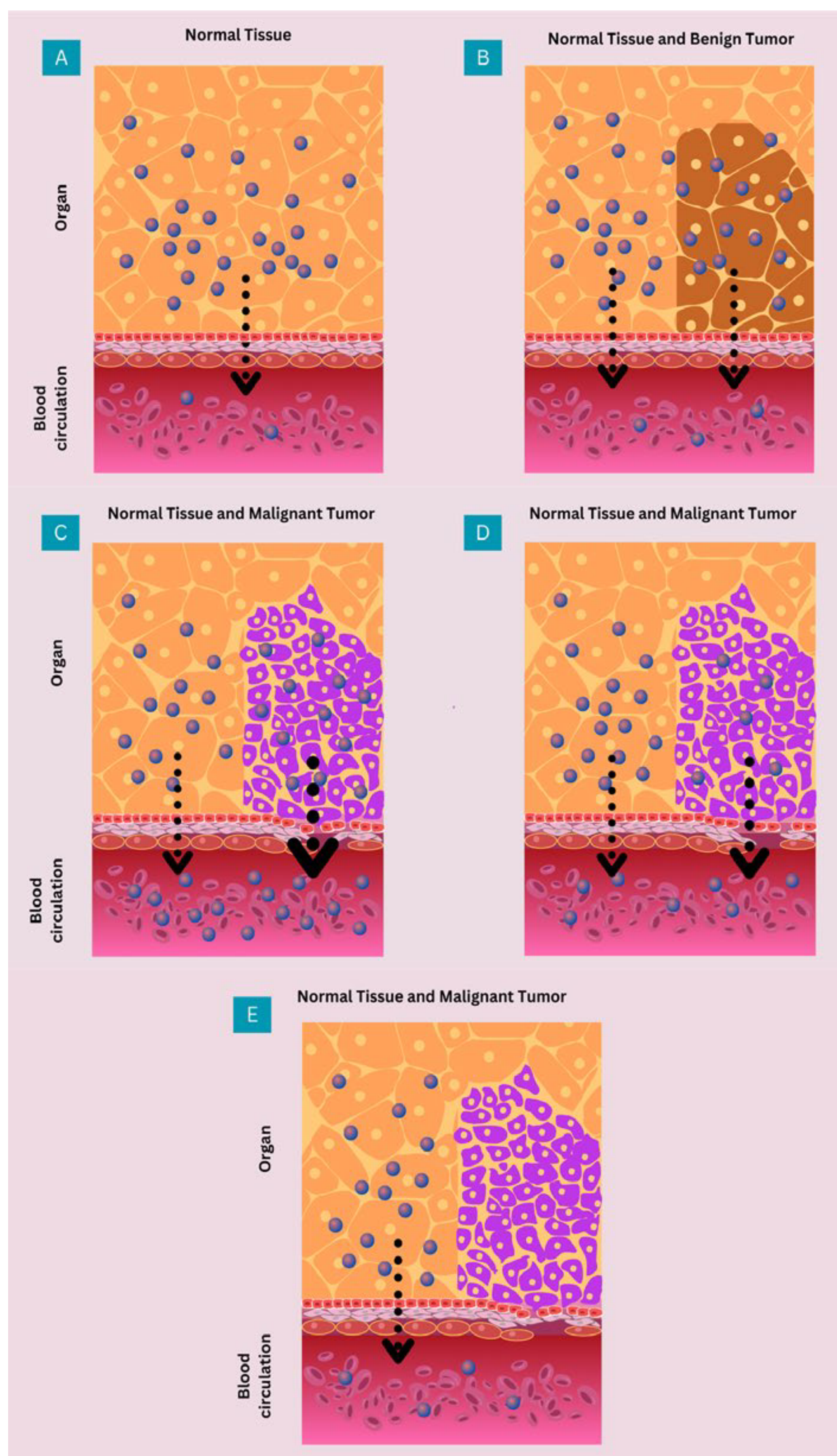
entered the clinic in the last 30-40 years. We and others have commented frequently on the failures of new cancer biomarkers to reach the clinic and identified numerous preanalytical, analytical and post-analytical shortcomings [2-4].

Among different classifications, cancer biomarkers can be grouped into two broad categories: those whose serum concentration is increasing in the presence and progression of malignancy (or as is alternatively stated "their concentration correlates with tumor burden") and those whose serum concentration presumably "decreases" with tumor presence or progression, in comparison to an appropriate non-diseased population. The first category of tumor markers is far more prevalent than the second one. We are not aware of clinically useful applications of circulating tumor markers which are inversely correlating with tumor burden. In this commentary, we speculate that many circulating tumor markers whose concentration is inversely related to tumor burden (i.e. their serum concentration decreases in comparison to controls when the tumor is first identified or is progressing) likely represent artifacts of the discovery process (false discovery) or epiphenomena. We further advocate that markers that decline with cancer progression should be carefully validated with well-defined groups of controls and patients, before definitive conclusions on their validity and clinical usefulness can be drawn.

The PSA paradigm

To illustrate our point, we will use the classical circulating prostate cancer biomarker, prostate specific antigen (PSA), as an example. In normal males, PSA is produced by the prostatic epithelial cells and is stored in the male reproductive system until ejaculation. The PSA concentration in seminal plasma is huge (~0.5 g/L) but only a minute fraction enters the systemic circulation, establishing a steady-state reference range of approximately 1 ug/L for adult males [5]. This is about a million times lower than the seminal plasma PSA concentration. Since normal prostatic epithelial cells and prostate cancer cells produce approximately the same amount of PSA on a cell-by-cell basis [5], the serum PSA concentration is not expected to be significantly altered when a patient develops prostate cancer. However, the sometimes-dramatic changes of serum PSA in prostate cancer patients (i.e. 100 ug/L or higher) are due to increased leakage of PSA from its vast normal reservoir (prostate tissue/seminal plasma) into the systemic circulation (Figure 1). There are numerous examples of tumor markers that increase in serum due to leakage from their respective, rich reservoirs (the contents of which normally, do not enter the circulation through physiological barriers). This mechanism of biomarker increase during disease state is similar to other commonly used non-cancer biomarkers, such as cardiac troponins; the latter increases dramatically in serum after myocardial infarction due to tissue damage/necrosis and the marker is released from its normal reservoir (cardiac muscle) into the circulation. Diagrammatic representations of a few scenarios are shown in Figure 1, which will be used for further discussion.

Figure 1: Diagrammatic representation of biomarker changes at various scenarios involving normal and cancerous tissues, as well as roughly equal biomarker expression or biomarker "downregulation".



The serum concentration of the biomarker will be dramatically increased only in Panel C. There is no circumstance in which the serum biomarker concentration will decrease below what is seen in non-diseased people. For more explanations see text.

Based on the information mentioned above, the increase of serum concentration of a biomarker at diagnosis (compared to controls) or disease progression, is easily explained (as shown in panel C). Biomarker leakage into the general circulation is the main reason for the observed increases during disease. As mentioned, biomarker decreases during cancer diagnosis (in comparison to controls) or progression, are rare in clinical practice and more difficult to explain. Most authors attribute such empirically observed serum biomarker decreases as biomarker "downregulation" in diseased tissues, which implies reduced transcription and/or translation of the biomarker in the disease (cancer) state in comparison to the normal state.

Panel A depicts a normal tissue (orange), acting as a reservoir of a (tumor) marker (blue dots), but the biomarker is not normally able to diffuse into the blood circulation due to the presence of physical barriers (epithelia, basement membrane, endothelia) and the tightly organized pattern of the prostatic cells. In such cases, the biomarker concentration differences between the reservoir (prostate; seminal plasma) and blood (red dots) can be as high as1,000,000-fold (as exemplified earlier by the PSA example).

Panel B depicts a benign tissue exhibiting biomarker expression per cell, roughly equal to that of the normal tissue. In this case, it is expected that the serum concentration of the biomarker will be modestly increased (2-3-fold) due to the larger amount of non-cancerous total tissue (normal tissue plus benign tissue). A good example of this is benign prostatic hyperplasia, whereby the size of the non-malignant prostatic tissue roughly increases by 2-3-fold. Panel C depicts a cancerous tissue exhibiting per cell, biomarker expression roughly equal to the adjacent normal tissue [5]. In this case, it is expected that the serum concentration of the biomarker will be dramatically increased due to leakage of the biomarker from the reservoir to the circulation due to the altered normal tissue architecture. In prostate cancer, the prostatic cells are disorganized and the layers between the prostate cells and blood vessels allow more PSA leakage into the circulation.

Panel D depicts a cancerous tissue exhibiting biomarker expression roughly equal to the normal tissue, but there is significant "downregulation" of the biomarker in the malignant tissue. In this case, the serum concentration of the biomarker is expected to increase, due to the additive effects of the biomarker originating from normal ad cancerous tissue. The biomarker increase will be higher if the biomarker is "upregulated" in the cancerous tissue.

Panel E depicts a cancerous tissue exhibiting total absence of biomarker expression. In this case the serum concentration of the biomarker is expected to be similar (but not lower) to the case of panel A, since the normal tissue will continue producing PSA while allowing a small fraction of PSA to diffuse into the circulation.

These examples illustrate that during cancer initiation and progression, the serum biomarker levels (assuming that the biomarker is produced by the tumor cells) are unlikely to decrease, even if in the cancerous tissue, the biomarker levels are generally "downregulated" or not expressed at all. One theoretical possibility is that the cancer cells may be inducing downregulation of proteins in normal tissue adjacent or distant to the cancer cells, during a process called "field cancerization" [6]. Otherwise, biomarker decreases in phase of cancer burden expansion should be viewed with caution and probably considered epiphenomena or false discovery. We are aware that there may be rare exceptions to our suggestion for certain tumors (pituitary carcinomas) where normal tissue destruction, coupled with the lack of biomarker production by the tumor, would lead to the decrease of the biomarker in the circulation [7].

Authors' Disclosure statements

MKC and EPD have no conflicts to report.

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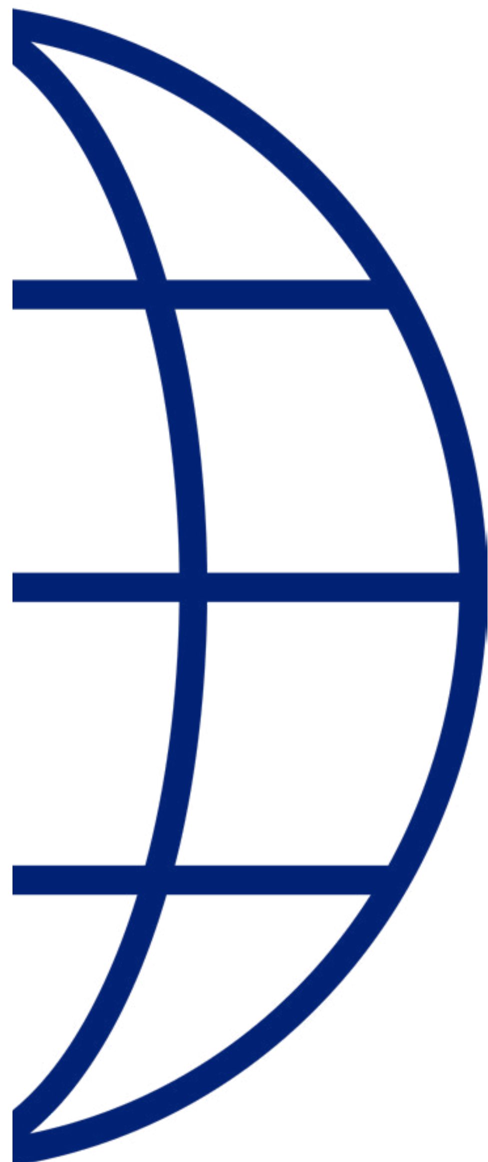

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