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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

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Foreword of the editor

Editor in Chief: Gábor L. Kovács, MD, PhD, DSc

Professor Ellis Jacobs is the chief of pathology, Henry J Carter and Coler-Goldwater Specialty Hospitals, New York and the associate professor of pathology at the New York University School of Medicine. He obtained his BSc in chemistry and natural science and defended his PhD in biochemistry in 1979. In those days he conducted research in the enzymology of oxidative phosphorylation. Professor Ellis Jacobs has a longstanding interest in point of care testing, laboratory consolidation and automation, computerization and automatic result reporting, and the impact on laboratory quality management systems and error reduction. His current research interests include immunodiagnostic assays, laboratory diagnosis of cardiac injury, laboratory management and automation, point of care testing systems, critical care medicine, toxicology and the application of informatics to improve laboratory efficiency. His current responsibilities include provision of management, leadership and vision for the Department of Pathology, Henry J Carter and Coler Goldwater Hospitals, directing all laboratory services, including point of care testing (POCT), overseeing quality assurance and quality improvement activities, teaching clinical pathology residents, and liaison with attending physicians, residents and house staff. Since 2011 Dr. Jacobs is also the director of Enzo Stat Lab, New York. He was elected to the chair of several task forces and committees in the American Association of Clinical Chemistry (AACC), Clinical and Laboratory Standards Institute, Clinical Ligand Assay Society, and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Within IFCC Dr. Jacobs chaired the Communication and Publication Division. Currently he is the liaison of IFCC and the representative on board to the Labs Are Vital program. He is editorial board member at eJIFCC and Pont of Care testing. Professor Jacobs published 62 scientific publications, many of them dealing with the role of laboratory medicine in acute care and POCT.

Professor Jacobs, the guest editor of the current themed issue of eJIFCC, has asked a number of renowned experts on the field of POCT to discuss current problems of this rapidly changing and increasingly important laboratory subdiscipline.

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Principles of point of care culture, the spatial care path™, and enabling community and global resilience

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Care path, customs, decision-making, empowerment, geographic information systems (GIS), geography, intervention, lifestyle, medical poverty, needs assessment, point-of-care (POC) technologies, POC testing, prevention, public health jurisdictions, small-world network, survey, and value

ABSTRACT

Goals: This article a) defines point of care (POC) culture; b) presents seven underlying fundamental principles; c) describes the importance of needs assessment; d) introduces a new innovation, the spatial care path™; and e) illustrates how POC testing that properly fulfills needs and spatial care paths™ enable community and global resilience.

Observations: Often, POC testing supplants the conventional clinical laboratory, which may be too distant, prohibitively expensive, or simply not available in limited-resource settings. New POC technologies "fit" future medical problem solving. Screening and testing directly in the home or primary care facilitate rapid diagnosis, monitoring, and treatment. In contrast to the past where attention has been placed on emergency departments, hospitals, and referral centers, the spatial care path™ starts with the patient and guides him or her through an efficient strategy of care in small-world networks (SWNs) defined by local geography and topology, long-standing customs, public health jurisdictions, and geographic information systems (GIS).

Conclusions: POC testing needs in limited-resource settings are striking. Fulfillment is best guided by thorough understanding of POC culture. Quick feedback and fast decision-making

by patients and physicians alike yield significant value that motivates changes in patient lifestyles and physician interactions. Culturally sensitive technology assimilation addresses leadership challenges in nations adapting to increasing populations of young and old, despite scarcity of resources. The spatial care path™ facilitates an essential balance of prevention and intervention in public health and shifts future focus to the patient, empowerment, and primary care within the context of POC culture.

INTRODUCTION—TERMS AND SCOPE

Broadly interpreted, *culture,* per se, has several practical definitions, including the beliefs, customs, and arts of a particular society, group, place, or time; a society that has its own ways of life; and a way of thinking, behaving, or working that exists in a place or organization.

We define *point of care culture* as medical empowerment of the individual and family nucleus integrated with norms, behaviors, beliefs, attitudes, expectations, POC technology, and outcomes (1,2). POC culture crosses the standard definitional dimensions of culture, because health is at the core of human existence, and people expect society to assure their good health. *Expectations* are strong beliefs that something will happen in the future. New technologies weigh heavily on expectations, and therefore, expectations should be assessed through needs assessment designed to improve health with POC testing.

POC testing is medical testing at or near the site of care (3). It includes *in vitro* testing with handheld, portable, and transportable instruments, as well as self-monitoring and noninvasive scanning. A *small-world network (SWN)* is a loosely tied and well, but not necessarily evenly, connected set of nodes in a scale-free network with a topology neither completely regular nor entirely random, such as roadmaps, extended families, and the spread of infectious diseases (4). A *geographic information system (GIS)* is a computerized approach that systematically helps organize point of need data in an electronic cloud for facile access, remote computations, and in the context here, decision-making in a medical GIS (5).

Skin autofluorescence (SAF), in the context of one of the instruments referenced here (Scout DS, Miraculins, Canada), is the measurement of light in the range of 360 to 660 nm from volar forearm skin excited with low-intensity multiple near-ultraviolet and visible wavelengths by light emitting diodes (LED) centered at 375, 405, 417, 435 and 456 nm (6).

FUNDAMENTAL PRINCIPLES—PREMISE AND HYPOTHESIS

First introduced by Kost et al. (1,2), the concept of POC culture is likely to become a focal point of future medical endeavors, in view of the monumental challenges of taking care of over 7.2 billion people in the world with the global population increasing at a rate of *several persons per second*. Technologies will be replaced perpetually with new inventions and innovations, but once arrived, and it is in the process of doing that, POC culture will forever stay as the new practice of medicine, actuated collaboratively by patients and physicians together.

Therefore, our fundamental premise is that POC testing will empower individuals to care for themselves in their own cultural context, despite burgeoning populations, diminishing resources, limited hospital access, and nations growing old before rich. In fact, patient self-management already exists. Patients with diabetes are taught to integrate self-monitoring and self-administration of therapy as part of their daily lives.

We hypothesize that increasingly, as people learn more about conditions that they can

prevent, they will prefer self-management of their own health. Thus, understanding POC culture means understanding the future of POC testing and how it can benefit people optimally. This article strives to outline several of the key principles of POC culture.

PRINCIPLE I—UNDERSTANDING HISTORICAL ROOTS

Table 1 (7-32) summarizes cultural principles related to medical care described by investigators through 2014. Additionally, Small et al. (33) argue that a study of poverty should be concerned with culture for scholarly and policy reasons. We devised an original demographic scoring system that showed the combination of a) poverty, b) insufficient health resources, and c) short supply of personnel who can perform diagnostic tests (e.g., medical technologists) identifies locations severely in need of POC testing (34). Once these settings have been defined, structured analysis can be set up to identify which POC tests to implement within the SWN (4). In these settings of significant deprivation of both materials and manpower, one must understand local culture well in order to alleviate POC medical poverty, which is one of the most important goals of cultural adaptation of POC technologies.

As we hit this milestone in history, that is, full recognition of the importance of POC culture, we will look back and realize that the world population was outstripping available health resources except in situations where foresight brought medical decision-making directly to points of need, wherever they might be—during birthing, in the home, for an emergency, or at a disaster. For example, The Point-of-Care Foundation, London, United Kingdom, focuses on the points of need for both patients and staff in terms of improving patients' experience of care and increasing support for the staff working with them (35). Similarly, the most recent papers in Table 1 illustrate further how medical professionals in several disciplines are recognizing the importance and impact of cultural expectations.

PRINCIPLE II—RECOGNIZING A DISRUPTIVE TRANSFORMATION

People everywhere must learn to take care of themselves, detect their own medical risks, and solve problems using new emerging POC technologies appropriately matched to therapies, including improvements in lifestyle and diet. Hence, mankind is evolving a worldwide transformative principle of enabling people with POC tools for personalized medicine at the individual level and in the communities where they live. These stronger communities will mean more resilient ones.

The key to this disruptive transformation is "point of care culture," that is, actual implementation of *medical empowerment of the individual and family nucleus integrated with norms, behaviors, beliefs, attitudes, expectations, POC technology, and outcomes.* Enlightened point of care tuned to local culture will enable a resilient future and help keep larger numbers of people healthy worldwide, despite disparities of disease, income, demography, and opportunity.

PRINCIPLE III—ASSESSING NEEDS

Expectations can be established through formal and informal needs assessment surveys which are addressed in detail in Kost et al. (36). See Kost et al. (37) for an example of a POC culture survey questionnaire. The overall cognitive process is summarized here in Figure 1. Subjects go through a series of cognitive stages when responding to surveys. After the interviewer presents the survey, subjects first interpret the questions and response options, retrieve relevant information from memory, and make

Table 1 Point of care culture—sociology, insight, and solutions

Sociological phenomena	Cultural insight	Point of care solutions and suggestions
Mindset that POC test results will have no effect on health outcomes & no future benefits	Religious belief that life outcomes are predetermined as well as lack of knowledge to improve health behaviors & lifestyle	Medical professionals can highlight how evidence improves health outcomes & connect with healthy eating habits & the importance of exercise
Some patients doubtful they can acquire necessary skills to utilize POC testing	Reliance on medical experts to handle all medical needs, significant level of illiteracy in older populations, & a belief that a high level of education is needed for device use	Medical experts should highlight the ease of using POC devices & use step-by-step free video teaching tools (e.g., YouTube) to instill confidence in patients
Fear that devices can cause physical discomfort	Belief that needles or injections given by oneself will lead to serious conflict	Reinforcement from others with similar backgrounds will dispel fear & mollify false beliefs
Conviction that devices are only for middle & upper class	Philosophical tenant that those with money are not similar to those with modest means	Highlight success stories of successful POC testing usage by poor people in rural areas
Inertia when faced with problems operating POC technologies, so patients drop long-term	Norms encourage not worrying about potential troubles & maintaining a relaxed mindset	Encourage medical volunteers to conduct home visits to prevent & mitigate POC technical problems in a timely manner
POC devices are inconsistently beneficial if medical professionals are unaware patients are using them	Medical professionals see numerous patients in a day, coupled with the fact that patients tend to play the role of spectator during physician visits	Tests, results, & trends in evidence should be integrated into the patient's medical record so the health team is aware of benefits for diagnosis, monitoring, & guided therapy at the point of need
Hesitancy to seek care for chest pain with delays in getting to the ER (7), plus language barriers	Stoic philosophy of the "poor" derived from engrained modest expectations & a life experience of suffering	Move screening for elevated cardiac biomarkers, such as cardiac troponin I or T, closer to the patient's home & into the hands of familiar people

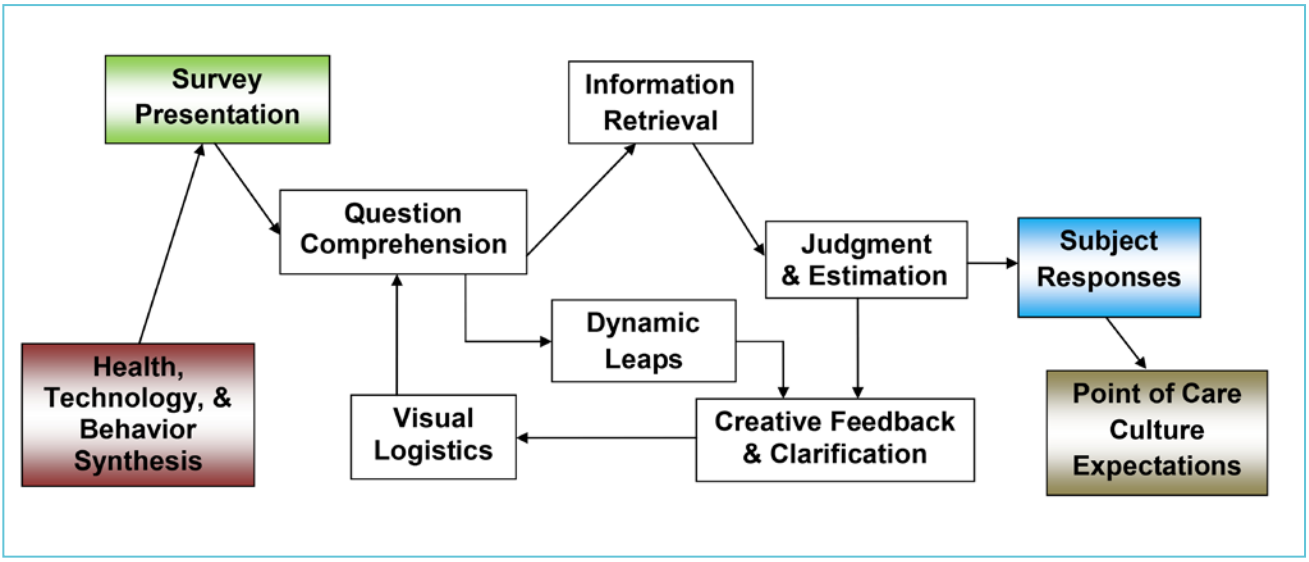
Sociological phenomena	Cultural insight	Point of care solutions and suggestions
Isolation experienced during the Great Bangkok Flood of 2011 (8)	High level of stress from lack of needed support by family & friends, & no acute care	Place POC devices on trucks carrying mobile labs above the water line, or in boats, & screen & rescue
HIV risk in border provinces due to human trafficking & lack of continuity of care among transient workers	Health screening is moving "private," whereby there is the potential for "hiding" diseases from public health scrutiny (9)	Provide POC tests to clinics, screen birthing mothers & newborns, use algorithmic testing routines, monitor viral load, treat more effectively, & prevent childhood morbidity & mortality
Dengue fever, malaria, & TB outbreaks & endemic areas where people must work outdoors	Public health problems may not receive adequate funding before the advent of resistant strains, when treatment becomes more difficult & risks higher	Invest in the future, invent new POC assays, focus on drug resistance, design platforms for novel approaches, & target therapy better
Broad teaching of healthy lifestyle habits may be ineffective for rural patients who are obese	Rural citizens may lack the creative ability to adapt teachings to their own life—they prefer a "how to" approach	Knowledge sessions (e.g., diabetes) must be specific & teach about glycemic index, portion control, & the effects of carbohydrates on glucose levels
POC devices can be less beneficial in certain patients over the long term	Needs assessment will reveal what each patient expects, why failures may occur, & how the care team can address them	Baseline data must be obtained for each patient (e.g., pre- & postprandial glucose) & individual trends in HbA1c followed quarterly (10)
Relationship between patient complexity, practice-level performance, & quality of rural care (11)	Proportions in diabetes control were lower for patients with greatest difficulty self-testing & keeping appointments—disjointedness	Reporting & resource allocation based on quality assessment must account for patient characteristics in vulnerable populations doing SMBG
Massive self-screening program (12) & urine alb:cr ratio testing (13)	Awareness of renal disease improved, & can assess risk & manage blood pressure better	Tab proteinuria overdiagnosis to be corrected with better color resolution, ACR better in community

Sociological phenomena	Cultural insight	Point of care solutions and suggestions
Roles for health care from the South-Isaan local wisdom in Khamer-Kui's Women (14)	Beliefs in local knowledge with traditional health care & treatment, holistic views based on epistemology, plus language & culture barriers leading to inaccessibility to governmental development	Position modern laboratory medicine, particularly POC testing & devices, to enhance health care delivery in the SWN & reassure the health status of people living there in terms of evidence-based medicine
Health beliefs & health care lifestyle of Thai-Song-Dam, one of the ethnic groups in Phitsanulok Province, in the present differ from the past (15)	Most of the Thai-Song-Dam people now access health facilities, such as sanitariums, clinics, & hospitals, compared to less use in the past, but many still have serious problems, such as diabetes & hypertension	Care paths should take into account not only acute & chronic diseases, but also the needs of different generations & changes with aging, so as to make continuous the POC culture of the future & to enlighten self-care
Self-testing for HIV, STDs, & cancer in different settings (16-18)	Cultural match varies resulting in successes & failures, & impact on population screening	Care paths must be culturally tuned & piloted before launching formal self-care POC programs
Neglected remote rural high prevalence population (19)	"Building Healthy Communities" proven with the introduction of convenient & rapid POC "one-stop" multidisciplinary services	Targeted POC tests provided multidimensional benefits in outcomes for patients with diabetes, "one-stop" multidisciplinary services raised awareness, enhanced community ownership, contributed to compliance, & helped doctor-patient relationships
Type 2 diabetes patients were more concerned about their personal perceptions of the outcomes they experienced related to complementary & alternative medicine use than to the opinions of health professionals (20)	Belief in their own experiences are stronger than the influence of external opinions	Medical professionals should provide POC test results to instill confidence in patients

Sociological phenomena	Cultural insight	Point of care solutions and suggestions
Rural elderly people have unsafe sociodemographic conditions & potential for low health care seeking (21)	Low economic status is one of the barriers in seeking medical care for morbidity	Place POC devices on mobile units that visit the elderly periodically, perform relevant testing, then counsel the elderly gently so that they believe in the evidence presented & will return for follow-up
Physicians & policy makers/ regulators had inadequate knowledge & negative attitudes concerning the proper use of opioids for cancer pain management in Thailand (22)	View that inadequate knowledge & negative attitudes represent real barriers	Provide sufficiently relevant training & give examples of modern POC devices accepted in hospitals worldwide for toxicology screening & detection of substance abuse
Caregiver dependent factors were more strongly associated with high burden than patient characteristics(23)	Age of caregiver, self-reported health status, self-reported income, & duration of care are associated with chronic diseases	Necessary, but easy to use POC devices (e.g., oxymeter & glucose meter) should be offered to caregivers to help reduce their burden
Thai Buddhist families' perspectives on a peaceful death in the ICUs (24)	The thought that "knowing death is impending" is important to families who prepare for & mange a peaceful death in ICUs	All related persons must arrive at a consensus regarding families' perspectives & POC trend monitoring may allay anxiety as families come to accept demise
Women's attitudes towards heavy menstrual bleeding & their impact on the quality of life (25)	Beliefs that heavy menstrual bleeding is problematic to social life, relationships, & work	Medical professionals should seek a proper way of mitigating heavy menstrual bleeding in women while also checking POC Hgb/Hct to avoid Fe-deficiency anemia
Community satisfaction with POC testing was validated using qualitative surveys of device operators in the Northern Territory, Australia (26)	Intangible qualities of POC testing can be as important to people as the underlying science	Analytical quality for POC testing met professional-based analytical goals & laboratory performance thresholds for most tests; >80% of respondents cited convenience & stated it assisted in the stabilization of patients with acute illness

Sociological phenomena	Cultural insight	Point of care solutions and suggestions
Delivering exemplary neurosurgical care in the future (27)	"Our profession is battling a relentless assault as numerous sectors implement change that impacts us & our community every day."	Consider adopting POC early detection technologies. "Innovation & diversity are crucial to encourage & reward when trying to effect meaningful cultural change, while appreciating the power of a 'Tipping Point' strategy will also reap significant benefits."
A jealous "widow ghost" (pii mae mai or lai tai) kills men in Surin, Thailand (28)	Superstitious evil spirit thwarted by "red shirt" postings outside houses thought to protect dwellers inside	POC EKG mobile monitoring to detect arrhythmia in Brugada Syndrome & avoid sudden death when asleep or ambulatory by continuous recording or telemetry
Education in cultural competency of radiologists in Japan (29)	Adapt practice specifically for Japanese behavior	Implement POC tests, such as rapid creatinine, to screen for renal compromise & reduce risks of contrast media
Ethical issues regarding information disclosure for consents in Saudi Arabia (30)	Male, post-procedure, & older patients are in favor of more information disclosure, while educated patients are particularly dissatisfied with current communications	Adopt point-of-need technologies, such as iPads with ample visual logistics, to communicate more effectively, & also perform bedside tests with immediate feedback enabling "physician capture" & fast feedback on status
Practice-based perspective on technology acceptance (31)	Innovation characteristics are reflected through the events of existing practices in the context of power-related concerns, resistance to change, & conflicts between professions in the creation of a new practice, namely, POC testing	The acceptance of an innovation is closely connected with the acceptance of existing & emerging practices, & key characteristics include reliability, speed, cost efficiency, usability, & ecology for POC testing approaches
Terror management theory & mortality reminders (32)	Examined if various mortality reminders would elicit more avoidant responses toward a novel device that indicates cardiovascular disease risk, the "CVD Risk Biochip"	Performance of initial qualitative investigations of the cultural worldviews of a particular cohort must come first, & the POC Biochip may have a beneficial effect on the potential uptake of screening behaviors, because it furnishes individuals with a risk status for developing a condition rather than indicating the presence or absence of a condition

Figure 1 Cognitive processes, flow, and stages experienced by subjects while being interviewed



After the interviewer presents the survey, first the subjects interpret the survey questions and the response options, then retrieve relevant information from their memories. Subjects then make judgments about the relevance and accuracy of their answers to the question and eventually report their response. Clarification by means of visual logistics assists the interview process and helps alleviate the "recency effect" by pointing, without biasing, subjects toward future decision-making.

a judgment about the relevance and accuracy of answers, then eventually report a response (38). Errors and biases can occur at each of the stages as a result of both internal and external factors.

It is worth noting that psychological theories and methods evolve rapidly and are constantly under debate. Nonetheless, Table 2 highlights key topics and valuable concepts for high quality surveys. Our POC culture survey, which is divided into POC testing, POC culture, and post-evaluation assessment, and can be found as Appendix 3 in *Global Point of Care: Strategies for Disasters, Emergencies, and Public Health Resilience* (37), aims to reveal underlying expectations, perspectives, beliefs, and acceptance of emerging technology, some of which is not yet available in the United States. Current knowledge of point of care and its technical and cultural attributes strives to integrate a logic web of future community and global resilience (39).

PRINCIPLE IV—CHARACTERIZING POINT OF CARE CULTURE

We investigated POC culture in rural Thailand by employing the principles above and composing questions for a 1-1.5 hour 1-on-1 survey (2,37). The survey was translated into the Thai language and approved by the Ethics Committee of Chulalongkorn University in Bangkok. Questioning of individual subjects was conducted by a native Thai-speaking interviewer familiar with laboratory medicine. When facing unexpected medical problems, subjects called for medical services mostly delivered at hospitals close to their home, reflecting confidence in and familiarity with local medical care, such as Primary Care Units, which are distributed near villages throughout Thailand, in contrast to services provided by university medical centers, which are quite distant and were least desirable. Interestingly, subjects were fairly receptive to services in homes, which bodes well for the use of POC testing and self-monitoring.

Table 2 Survey characteristics that produce objectivity, quality, and impact

Neutral survey presentation	***Elimination of false inferences that result from biased survey delivery.*** If the respondent's perceived intent of the survey is different from the researcher's actual purpose, the respondents are likely to only provide information that they believe the interviewer is seeking, which could be inconsistent with the actual purpose of survey.
Question comprehension	***Avoidance of comprehension errors that affect data quality.*** Sources of comprehension errors include ambiguity, low-frequency words, vague quantifiers, and excessive complexity.
Memory retrieval	***Reducing retrieval failure.*** Different forms of retrieval failure include memory decay, interference, and distortion. Researchers should avoid asking overly detailed questions, allow subjects to review past records, increase respondent time on tasks, and provide retrieval cues. ***Minimizing the possibility of availability heuristic.*** Availability heuristic is a mental shortcut used when one estimates frequency or probability of events by the ease with which instances could be brought to mind rather than examining other alternatives. The result may not reflect the actual probability of the events happening. ***Elimination of the priming effect.*** Exposure to certain stimuli may trigger activation of related thoughts later. For example, when exposed to the notion of diabetes, concepts like disease, blood glucose, and insulin are activated as well, making them easier to be retrieved than other concepts. Careful ordering of questions can help reduce priming effect.
Judgment and estimation	***Avoidance of satisficing.*** Difficult tasks, lack of motivation, and limited capability tend to make the respondent only search for a satisfactory choice rather than the best alternative. Simplifying the tasks so that respondents are capable of executing the task and gaining cooperation from respondents are effective solutions. ***Elimination of acquiescence bias.*** Acquiescence bias refers to the tendency for respondents to agree with a statement, which usually happens with agree/disagree questions. One solution is to use item-specific response options.
The recency effect	***Balancing recent and past events.*** There is a tendency when making decisions to give recent events more weight than things further in the past. Visual logistics can point the subject forward, rather than backward, and encourage responses in synch with future decision-making.
Subject response	***Elimination of social desirability bias.*** Respondents tend to deliberately misreport their behaviors in order to be viewed in a positive light. Researchers can control the interview mode and survey wording to minimize social desirability bias.

When questioned about preferences for medical care, subjects preferred quick diagnosis and treatment, friendly staff, availability of technology, and short time to reach help, in that order. Oddly, quality came in last. The survey tool includes several questions and "visual logistics." These illustrations help simulate actual interfacing with diagnostic testing, which is not performed during the questioning period. The subjects thought that capillary blood sampling would be the most uncomfortable procedure, followed by venipuncture, while innocuous collection of urine samples and noninvasive scanning were not deemed uncomfortable. Since capillary blood sampling is common to POC testing and "nanotainer" microassays (40), we will investigate subject past experiences further to determine if lancets were selected inappropriately, fingertips were pierced several times, or collection methods were performed incorrectly in the absence of adequate training, which one author (GK) has observed several times in the field. Nonetheless, subjects recognized the merit of noninvasive scanning.

Subjects felt that the fear of the procedures, views in their community, and potential personal risks from procedures influenced their choice of medical care. Religious beliefs, government policy, family opinions, and superstition occupied the middle ground, while the costs of care were not deemed that important, possibly because they had so-called 30 Baht (~1 USD) "gold cards" that gateway inexpensive access to care. Other responses revealed attitudes toward and knowledge of diabetes (see below). In short, the survey tool provided an efficient approach for exploring the basic characteristics of local POC culture, namely, norms, behaviors, beliefs, attitudes, expectations, POC technology, and outcomes, and we intend to continue this discovery process in order to integrate effective therapy, which may be as straightforward as lifestyle and diet changes that will reduce risk of diabetes.

PRINCIPLE V—REACHING OUT AND EDUCATING

Common diseases, such as prediabetes and diabetes, must be addressed by modifying beliefs, habits, lifestyles, diets, and knowledge, while implementing novel instruments that redefine medical self-sufficiency at the point of need, and do it meticulously, individually, and socially within communities on a global scale. While POC culture is strikingly multifactorial (1,2), it is the element of *expectation*, per se, that has changed medical transactions and confronts physicians, nurses, and care teams every day, since POC testing is virtually ubiquitous throughout the world, and people expect immediate knowledge, diagnosis, problem solving, and treatment, despite circumstances that may be beyond the ability of the healthcare system to control (e.g., newdemics), while still delivering high quality care at points of need.

As POC information becomes organized into collective SWN knowledge in the accessible GIS, the future impact of POC culture will become apparent to public health practitioners, strategists, and importantly, businesses, the practical providers of the new and emerging technologies. Culturally, with a philosophy of promoting self-care for both personal and public health, one must know how patient behavior affects POC results and vice versa. For example, some POC results, such as evidence of HIV-1/2 and Hepatitis B (or C) infection, may have devastating immediate impact on patients and their families.

Therefore, test results should be presaged by outreach and education, a theme we encourage. In rural limited-resource communities, major challenges arise when linking self-testing with treatment in a manner whereby the

patient, the primary care nurse, the physician, and the pharmacologist all agree long-term and then see to it that there is persistence, continuity, oversight, and correction of non-compliance. Thus, development of care paths in local languages will facilitate cost-effectiveness, improvements in outcomes, and future cultural acceptance. We call this the *final frontier* of POC testing (1,2)!

PRINCIPLE VI—CREATING COMMON PURPOSE IN PUBLIC HEALTH

While technology plays an important role in disease management at the point of care, thorough understanding (the compass) of local culture is just as crucial. Both a map (needs assessment results) and compass are necessary to arrive at medical destinations successfully, both literally and figuratively. Effective utilization of needs assessment surveys, which requires sufficient understanding of survey psychology (2, 37), can provide healthcare professionals with invaluable information about local customs, values, and lifestyles to build a strong foundation for a targeted, interactive, and meaningful POC program.

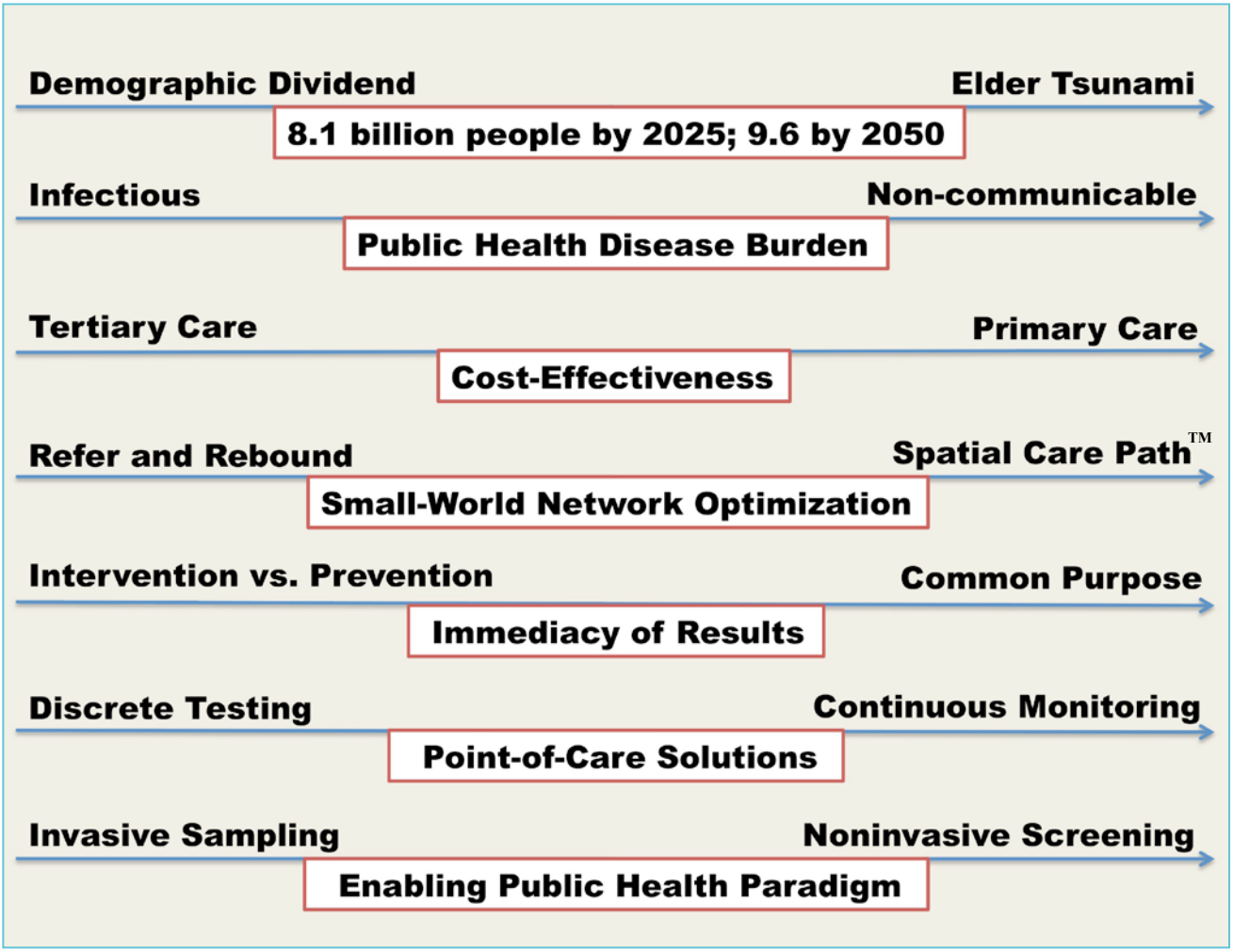
Particularly important cultural aspects worth exploring include dietary habits and preferences, religious beliefs, individualism/collectivism, degree of long-term orientation, power distance index (the extent to which patients, as less powerful participants in the healthcare organizations, accept and expect that power is distributed unequally), and attitudes and preferences regarding different POC technologies before deciding which to implement. As more and more handheld and portable devices are implemented, they will become second nature, possibly to the extent where the "point of care" concept, per se, is nearly forgotten, and personal diagnosis and monitoring become routine, commonplace, and because of economies of scale, inexpensive.

Professionals should strive for continuous collaboration between laboratory medicine and other fields, such as psychology and public health in order to develop successful culturally tuned POC paradigms for the future. As medicine moves progressively to points of need, as it must in order to be cost-effective in overly crowded societies, people increasingly will make their own decisions about which devices and modalities to select for self-care. Therefore the power index will shift in favor of the patient, who becomes, for all practical purposes, a public health practitioner.

Just like it is almost unreasonable not to be able to contact anyone anywhere on a cell (mobile) phone nowadays, POC will do the same, but with simultaneous risk assessment and diagnostic information, which will be distributed and assessable on site. Marketplace competition will speed that process, both for patients and physicians. Business entrepreneurs will see to it, in part because this new approach will improve the health of their employees. Ideally, the evolving strategies, decision-making, and value propositions will be informed, guided, and seasoned.

Kost LK et al. (41) devised such a scheme for risk assessment and diagnosis of prediabetes in India. The approach, which integrates prevention and intervention to create common purpose in public health, is summarized briefly here. Current global perspective suggests that there is convergence to a single common purpose in the public health field as non-communicable diseases displace infectious diseases in terms of the vast numbers of people afflicted, and ageing of world populations increases the prevalence of non-communicable diseases while disproportionately adding chronically ill elderly to societal burden (Figure 2).

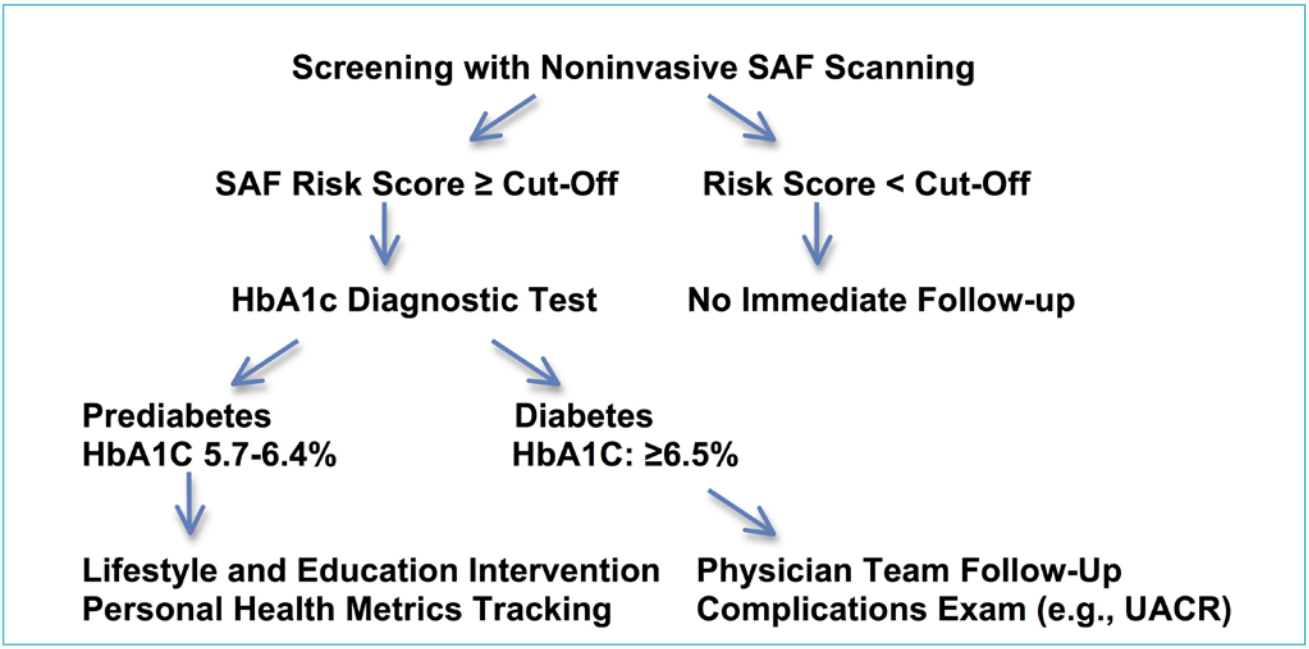
Figure 2 The vectors of future public health



Seven vectors will strongly influence the future destiny of public health. They reflect inevitable changes transforming current professional practice to a paradigm that better fits ageing societies burdened with non-communicable diseases. To achieve cost-effectiveness, innovative POC solutions are necessary at the primary site of care. Patients follow spatial care paths™ in optimized small-world networks without inefficient hospital referrals and rebounds after discharge because of lack of personal ownership of solutions that facilitate preventative health measures, such as improvements in lifestyle, diet, and physical activity. POC screening, testing, and monitoring provide immediate evidence-based results for simultaneous prevention and intervention. The program plan for prediabetes uses noninvasive screening and POC HbA1c testing of capillary fingerstick blood samples, an innovation that is enabling to the new public health paradigm.

The program plan comprises evidence-based metrics [body mass index (BMI), skin autofluorescence (SAF) score, POC HbA1c, sugar-sweetened beverage (SSB) consumption, questionnaire follow-up, physical activity (steps walked determined by pedometer), and an optional POC lipid panel for the assessment of metabolic syndrome] of patients with prediabetes discovered during an initial subject encounter that occurs at the place of employment or other convenient location for the individual being screened. Then, the test cluster of risk, diagnostic, and potentially therapeutic data are gathered rapidly and on site. Figure 3 shows the decision tree. Thus, we anticipate that public health will be transformed to blend prevention and intervention in one common purpose (Table 3).

Figure 3 Prediabetes program flowchart



One of the primary advantages of the program plan designed as a community approach is that large numbers of subjects can be screened quickly, painlessly, and cost-effectively to discover those with prediabetes who merit further evaluation, entry into the education branch of intervention, and follow-up with personal health ownership and outcomes metrics that track successful personal management and a return to healthy living. [UACR is urine albumin to creatinine ratio.]

Table 3 Factors transforming public health: Point of care will merge intervention and prevention

Decreasing birth rates and increasing longevity are reshaping disease patterns from infectious and acute to non-communicable and chronic
Internationalization of medical science, globalization of the healthcare workforce, and convergence of common purpose are transforming standards of care
Globalized economies bring hazards, set-backs in lifestyles, and need for international disease control in the local context
Local patient encounters take place in the context of worldwide information access and influence, so likewise, access to public health must be reengineered for the point of care
Outcomes measured by objective metrics will increasingly depend on assimilation of appropriate POC technologies at dynamic points of need
Spatial care paths™, that is optimized treks through small-world networks, are facilitated by POC screening, monitoring, and testing, which simultaneously merge intervention and prevention to create common purpose in public health

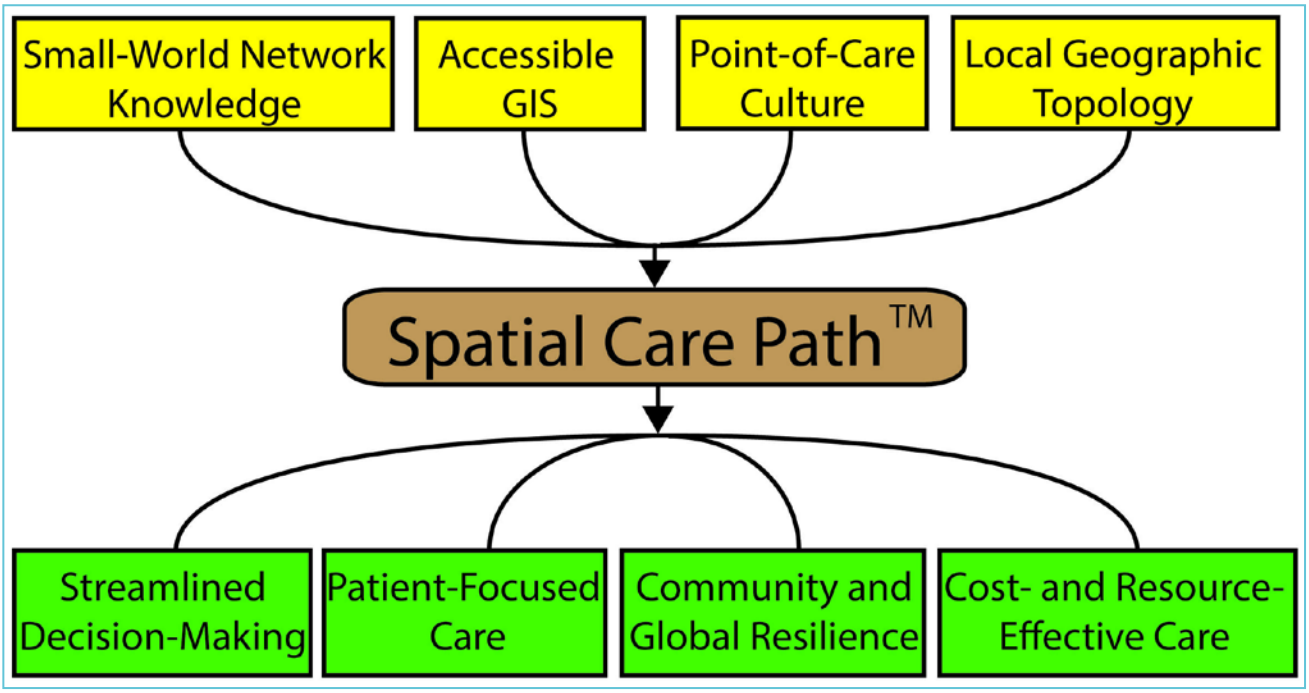
PRINCIPLE VII—INNOVATING THE SPATIAL CARE PATH™

Definition and utility

We define the spatial care path™ as the most efficient route taken by the patient when receiving definitive care in a small-world network (SWN). While being introduced formally here and also recently for the first time at a national meeting in the United States (42), the spatial care path™ concept eventually will dominate the delivery of healthcare as populations expand, the elderly increase in numbers, and common sense, not to mention financial necessity, dictates that care must shift upstream to the site of the patient in order to conserve resources, save time, and spare lives (Figure 2). In other words, just like POC testing, it will become commonplace in future POC culture for the patient to increasingly take possession of his or her own medical destiny (read: screening, diagnosis, monitoring, and therapeutic adjustments) and by doing so, the collective community will benefit from enhanced resilience.

The inputs and outputs for a spatial care path™ (Figure 4) are self-evident—local geographic topology builds the physical relationships between the community and their health resources; SWN knowledge helps define how these communities relate to and utilize these physical connections, and identifies inefficiencies; POC culture helps distinguish gaps in knowledge or cultural barriers inhibiting technology adoption; and a GIS ensures that this information is accessible at the point-of-need. The end result of this analysis is that POC testing will be implemented at relevant locations that streamline decision-making, enable patient-focused care, improve overall community global resilience, and reduce costs and resource utilization providing more effective and efficient care.

Figure 4 Logistics of knowledge processing by the spatial care path™



A spatial care path™ models existing infrastructure and suggests improvements that result in streamlined, patient-focused, cost- and resource-effective, and resilient care.

Key features

Key features of a spatial care path™ include but are not limited to the following: a) it starts with the patient wherever the patient is located, rather than at the other end of the spectrum, that is, at the medical institution; b) the patient progressively participates in decision-making by virtue of self-monitoring and POC tests available beginning in the home, primary care, and SWN hubs; c) it is highly facilitated by POC testing in that distributed diagnostics provide timely evidence-based decision-making along the way; d) it establishes access to the most critical elements and scarce specialists of the healthcare delivery system in the SWN; e) it can be managed quantitatively by means of the real-time GIS, the POC test vector, V_{POC}, and the access vector, V_{ACCESS}; and f) it is particularly useful in limited-resource settings because it optimizes the use of medical resources within the SWN, especially with the SWN becomes compromised or isolated by natural disasters, complex emergencies, or pandemics and necessary quarantine.

The role of the Geographic Information System (GIS)

A spatial care path™ relates populations and the resources that provide care. Since these relationships are linked to geography, it makes senses to evaluate them in that context. Geographic information systems allow one to view and analyze spatial relationships among populations, resources, road networks, and other attributes (6,43,44). Using a GIS to explore spatial care paths™ allows one additionally to visualize inefficiencies inherent within SWNs and model alternative POC placement schemes that will streamline access to care. The ability to visualize the SWN helps understanding of the advantages of POC technologies before implementing them in the real world, thereby saving resources, time, and money, and at the same time, establishing resilience within the SWN.

Modeling population health access within a spatial care path™

Evaluating the spatial care path™ involves modeling population health access to diagnostic resources and then care. The spatial care path™ provides a structured analysis through a GIS of geographic entities (e.g., roads, hospitals, and population locations), SWN culture phenomena, and diagnostic resources to provide an objective analysis of appropriate changes to health resources. This spatial model provides a means to evaluate how to integrate POC to identify the critically ill and streamline their transportation to appropriate resources.

For example, exploring how integrating POC into different levels of the health system (e.g., primary care, community hospital hubs, and referral or tertiary care centers) or ensuring all population are within 60 minutes of a diagnosis, could set policy strategies for health officials. The GIS can then identify the most effective placement of POC technologies to prove evidence-based and streamlined care consistent with those policies.

Translating delivery systems into spatial care paths™

Care paths for specific diseases exist, or are being formulated to understand the best way to deal with adverse sequelae and downstream complications for which care by specialists is very expensive. For example, a diabetes care path was developed for rural Isaan in Thailand (45). The care path starts with primary care close to where patients live. Thus, in some cases the spatial care path™ can start with the diagnosis of preconditions, such as prediabetes by means of the strategy in Figure 3, and allow people to adopt changes in their lifestyle before prediabetes evolves into diabetes. These actions

improve health and decrease costs before more drastic complications appear. Disease-specific care paths serve as a good media to be translated into dynamic spatial care paths™. By understanding where patients are physically located through the GIS, inefficiencies can be observed upstream and alternatives can be modeled that will accelerate SWN operations downstream.

Spatial problem solving

A novel example of the dynamic spatial care path™ is provided by the efficacious approach to the acute rescue of Aboriginal Australians with acute coronary syndromes (ACS) (46). The Australian program was motivated by discrepancies in mortality rates of underserved peoples in rural areas of Australia who did not fare as well as their more affluent peers in metropolitan areas, and for whom "…distances to PCI (percutaneous coronary intervention) centres exceed 250 km…." The executive cardiac specialist on call for the integrated cardiovascular rescue network benefits from telemetry of raw data provided by the actuation elements of V_{POC}, which is established with proper quality control and other proactive enhancements to include essentials, such as the ECG, cardiac troponin T, and electrolytes, obtained at the point of care. Risk stratification is achieved with POC cardiac troponin testing. Operationally, V_{POC} becomes a risk assessment vector.

In the Australian program, a pivotal decision occurs when the physician lead decides whether or not evidence is adequate to order fixed wing aircraft rescue (at a cost exceeding $2,000 USD). Signs and symptoms of acute myocardial infarction (AMI) must be documented rapidly and adequately to warrant immediate coronary catheterization or other life-saving measures not available in the rural area. Program successes include relieving rural areas of unnecessarily high death rates from AMI, providing equity to Aboriginal Australians who previously did not receive optimal interventional cardiac care, and other public health benefits such as improved quality-adjusted life years.

Specifically, "…availability of immediate cardiac support was associated with a 22% relative odds reduction in 30-day mortality…" and "… lower mortality (was) observed among transferred patients." In this case, the primary diagnostic work-up of the patient at the point of need, access to POC cardiac biomarker testing in V_{POC}, the fixed-wing aircraft, and the case resolution in the hands of highly trained specialists at the referral hospital represent some of the key edges (process steps) in the real-time spatial care path™, which is optimized for each patient episode in the context of the geographic SWN, its topology, and net inaccessibility, which is overcome with the rescue flight. Therefore, this example of the spatial care path™ starts with the patient, evidence, and interpretation at the point of origin, and then moves the critically ill patient toward intervention, thereby optimizing strategy, sequence, and outcome.

CONCLUSIONS AND RECOMMENDATIONS: PROPELLING A FUTURE VISION

- Research findings generated by surveying POC culture motivate the sound practice of POC testing, thereby enhancing the efficacy and effectiveness of diagnostic tests and disease-specific care paths.
- Lifestyles, attitudes, expectations, and beliefs influence decisions on adopting current screening methods for prediabetes positively, and thus, noninvasive screening and associated POC testing might provide sufficient knowledge to forestall increases in prevalence.
- Integrative initiatives, such as an holistic approach recommended above for prediabetes discovery, management, treatment, and follow-up, conducted simultaneously with

lifestyle changes and consumption of nutritious diets, represent vital building blocks for meeting future economic challenges in nations lacking adequate healthcare resources, yet at risk of growing old with the added burden of expensive disease complications before growing rich.

- Culturally aware POC solutions should be tuned to local societal norms and characteristics in order to optimize effectiveness, and readers can take advantage of the survey provided in Appendix 3 (37) of *Global Point of Care: Strategies for Disasters, Emergencies, and Public Health Resilience* as a starting point for investigation and exploration.
- Metric value scales can be established from survey subject preferences, such that a POC test will support clinical decision-making within its operational context of local culture, and therefore, evaluation metrics will be more "organic" for emerging POC technologies than for conventional diagnostic tests performed in the hospital clinical laboratory.
- Unmet needs assure a bright future for inventors, innovators, and entrepreneurs who develop these new POC solutions, that is, new elements of V_{POC}, such as SAF screening delivered reagent-free and inexpensively at the site of care for rapid detection of diabetes and cardiovascular risk, and therefore, government and private sectors should draw public attention to the high quality and cost-effectiveness of noninvasive screening and other promising newcomers.
- As small, connected, and handheld POC devices and smartphone diagnostics become ubiquitous throughout society, a new way of living and thinking that embraces ownership of healthcare will become natural—part of everyday human existence that is highly informed.
- Spatial care paths™ provide a structured analysis of health attributes in the SWN and model how to make it meet the demands and needs of the community.
- Harmonizing diagnostic testing in SWNs will accelerate progress in the ASEAN member states, China, and other countries and their limited-resource rural areas by improving the accessibility, quality, usefulness, and impact of POC test results.
- Practicing point of care in the context of local culture represents the *final frontier*, and if explored successfully, will become a notable achievement for the 21st Century!
- Ultimately, POC culture will be recognized as one of the most important characteristics for reducing medical poverty, and its understanding will create impactful solutions and resilience at points of need worldwide.

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Risk management for point-of-care testing

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ABSTRACT

Point-of-care testing (POCT) is growing in popularity, and with this growth comes an increased chance of errors. Risk management is a way to reduce errors. Originally developed for the manufacturing industry, risk management principles have application for improving the quality of test results in the clinical laboratory. The Clinical and Laboratory Standards Institute (CLSI), EP23-A Laboratory Quality Control based on Risk Management guideline, introduces risk management to the clinical laboratory and describes how to build and implement a quality control plan for a laboratory test. A simple, unit-use blood gas analyzer is utilized as an example for developing a laboratory quality control plan. The US Centers for Medicare and Medicaid Services (CMS) has revised the Clinical and Laboratory Improvement Amendments (CLIA) interpretive guidelines to provide a new quality control option, individualized quality control plans (IQCP), for decreasing the frequency of analyzing liquid controls from two levels each day of testing to manufacturer recommended frequencies in conjunction with a device's built-in internal control processes and the risk of error when testing with that device. IQCPs have the advantage of allowing laboratories the flexibility to adopt alternative control processes in concert with traditional liquid controls to improve efficiency and cost effectiveness while providing optimal quality POCT results for patient care.

INTRODUCTION

Point-of-care testing (POCT) is an increasingly popular means of delivering laboratory tests close to the patient. POCT allows for rapid diagnostics and turnaround of test results to provide for faster medical decision-making and improved patient outcomes (1). However, if inappropriate samples are collected, specimen is mislabeled, analysis is performed incorrectly, or test is misinterpreted, wrong results may be reported and acted on by the clinician. Studies have indicated that for central laboratory testing, most errors occur in the preanalytical phase, prior to the sample arriving in the lab (2). For POCT, the majority of errors occur in the analytic phase of testing (3). In fact, errors can occur in any phase of laboratory testing whether performed in a central laboratory or at the point-of-care. As laboratory directors, we should know our processes and take steps to detect and prevent errors before those mistakes reach the clinician and affect patient care.

Risk management is a way to reduce errors with POCT. Risk management is defined as the systematic application of management policies, procedures, and practices to the tasks of analyzing, evaluating, controlling, and monitoring risk (4). Risk is the chance of suffering harm or loss. Risk is generally assumed from the patient's perspective, but risk can also apply to the operator of the POCT device, the laboratory administration and even the hospital and its reputation. Risk is the chance of suffering harm or loss, and risk can be estimated through a combination of the probability of occurrence of harm and the severity of that harm(5). Errors that occur more frequently have greater risk, and errors that lead to greater harm also present greater risk. So, there is a spectrum of risk from low to high. Once can never get to zero risk. There is always some chance of risk. Our job as laboratory staff is to maintain risk to a clinically acceptable level.

Risk management should not be a new concept. Laboratories conduct a number of activities to limit their chance of errors. The performance of new tests is validated before use on patients. Staff troubleshoots control failures and follows-up on complaints from clinicians. When errors are detected, the harm to patients is estimated and actions are taken to prevent recurrence in the future. So, risk management is simply a formal term for many of the activities that laboratories are already doing.

QUALITY CONTROL

Quality control is a means of detecting and preventing errors. Besides frequency and severity, detectability is a third factor in the risk estimation equation. Quality control and risk management principles were developed from the manufacturing industry. As products are constructed on a factory line, the quality of the product is inspected to ensure that it meets manufacturer specifications. If problems are noted, the line can be stopped and production corrected to ensure the quality of the final product.

These industrial risk management principles have application for reducing errors in laboratory testing as well. In the central laboratory, batch reagents are used for several days. During that time, the regent can drift and degrade impacting the test result. Laboratories analyze liquid quality control, a stable sample with predefined acceptability ranges, in order to detect reagent problems before they affect the test result. Traditionally, liquid controls are analyzed at two concentration levels each day of testing or more frequently as required by the stability of the test system.

Liquid quality controls do a good job at detecting systematic errors. These are errors that affect the patient sample in the same manner as the quality control sample. Reagent degradation, calibration errors, dilution and pipetting

errors are examples of systematic errors that quality control can effectively detect and prevent before the errors affect a patient result. Quality control, however, does a poor job at detecting random errors which uniquely affect individual samples. Bubbles, clots, drugs, hemolysis and other sample specific errors are not detected by liquid quality control. Other mechanisms, like bubble and clot detection, or analyzer hemolysis indices must be utilized to detect random errors. So, analyzing two levels of liquid quality control each day of testing does not entirely eliminate risk, and laboratories have still produced bad results despite analyzing liquid quality control.

Newer POCT devices utilize unit-use cartridges or test kits. Analysis of liquid quality control consumes the entire test in the process, and there is no guarantee that the next test will perform identically. Alternative control processes must be used for these devices in addition to liquid controls in order to optimize the quality of these tests. Many POCT kits have built-in biologic and chemical controls to ensure the performance of individual tests. Fecal guaiac occult blood cards have a positive and negative control area on each card to ensure the reactivity of the card and developer. Urine pregnancy tests have a control line on each test to verify test storage and viability of the antibodies on the test. Drug, rapid strep, HIV and other POCT unitized tests have similar control lines or regions that guarantee the quality of the test kit and result with each test.

These control lines are control processes that act as an alternative to traditional liquid controls in order to detect the risk of specific errors when using those tests. Some tests, like bilirubinometers, cannot even accept a liquid sample, so alternative control processes must be utilized to ensure the quality of this test. Consider molecular testing where hundreds of reactions may occur on a single chip. How does a laboratory effectively control the quality of these tests? It is neither economical nor possible to analyze two levels of liquid controls for every reaction on this test each day. The effective way to ensure quality would consider risk of those errors that are most likely to occur or cause greatest severity of harm from an incorrect result. The amount and quality of specimen, the reactivity of the replicating enzyme, and the thermocycling of the device are key failure points, and those are the steps that should be monitored by the quality processes.

Laboratories must partner with the manufacturer to develop an effective quality control plan. Although the practice of analyzing two levels of liquid quality control have given laboratories some degree of assurance that results are valid, newer devices have built-in electronic controls, and on-board chemical and biological controls, No single quality control procedure can cover all devices, since devices may differ in design, technology, function, and intended use (6). Quality control information from the manufacturer increases the user's understanding of device overall quality assurance requirements, so that informed decisions can be made regarding suitable control procedures. Manufacturers understand their devices and the limitations of those devices, while laboratories know how the device will be utilized and test results applied for patient care. A quality control plan identifies the weaknesses in the testing process and defines the roles of the manufacturer built-in control processes and laboratory actions required to maintain risk to an acceptable level.

The Clinical and Laboratory Standards Institute (CLSI) document EP23-A introduces the industrial risk management principles to the clinical laboratory (7). EP23 describes good laboratory practice for developing a quality control plan based on manufacturer's information, applicable regulatory and accreditation requirements, and the individual healthcare and laboratory

setting. This guideline recommends collecting information about a test system and processing that information through a risk assessment to develop a quality control plan. The testing process is mapped from preanalytic through analytic and postanalytic phases. Weaknesses in the testing process are identified and for each hazard identified, the laboratory defines a control process which will detect and prevent that error, controlling risk to a clinically acceptable level. Some hazards, like use of expired reagent, may be effectively controlled through a manufacturer built-in process such as barcoding which prevents the operator from utilizing expired reagents. Other hazards may require the laboratory to take an action, like instrument maintenance or operator training/competency. A quality control plan is essentially a summary of all the hazards considered and laboratory actions required to minimize risk. Once developed, the quality control plan is implemented and monitored for effectiveness. If errors continue to occur, the laboratory is encouraged to troubleshoot, reassess their risk and modify the quality control plan as required.

RISK MANAGEMENT EXAMPLE

A unit-use blood gas device may be used as an example of the risk management process. The first step to build a quality control plan is to collect information about the test. Let's consider a generic POCT blood gas and electrolyte analyzer intended for use in a same-day surgical center. The need for testing is low, only 1 – 2 tests per day. At a cost of $10 – 20 per test, the requirement to perform two levels of liquid control each day of testing will increase the cost of testing significantly and add to the turnaround time of results since control results will need to be evaluated before patient testing can be conducted. The use of alternative control processes provided by the manufacturer will improve cost, test and labor efficiency.

Review of the package insert allows the laboratory to determine intended use, test system operation and test limitations. The system is a portable clinical analyzer for the *in vitro* quantification of various analytes in whole blood. The test system consists of the portable clinical analyzer, test cartridges sealed in a foil pouch for protection during storage, quality assurance materials (liquid control and calibration verification solutions), and a data management system with a server class computer, data management software, wireless connectivity, and laboratory and hospital information system interfaces. The unit-use cartridge contains all the components to perform testing including; a calibrant solution, reagents, sample handling system, and sensors. The analyzer automatically controls all steps of the testing process such as fluid movement, calibration, fluid mixing, and thermal control. The cartridges are standardized to plasma core-laboratory methods using multi-point calibration curves stored in the device memory that are stable over many lots. Upon insertion, a calibrant solution in the cartridge is passed over the sensors. Signals produced by the sensor responses to the calibrant solution are measured, and a one-point calibration adjusts the sensor offset to the stored multi-point calibration curve. The analyzer then moves the sample over the sensors and the signal of the sensor responses to the sample are measured from the adjusted calibration curve.

Examination of the manufacturer, internal control processes allows an understanding of how the process functions and what errors can be detected and prevented with that process. The blood gas and electrolyte analyzer contains simulated internal control processes that check the edge connector, internal electronics and analyte circuitry. The internal control simulates the electronic signals that are produced during a cartridge test. An isolated region of the internal circuit board sends a range of simulated sensor

signals through the cartridge measurement channels. The range of signals encompasses the entire linear range expected from blood analytes. Next, conductivity out of the connector pins is measured, insuring no contamination is present on the edge connector which would interfere with the test. Signal measurements must fall within strict predetermined thresholds in order to pass. The internal simulated control is performed automatically every 8 hours or if there has been a significant change in analyzer temperature, from cold to hot, since this can cause condensation on the connectors. The internal control can also be performed manually whenever the performance of the device is in question. Internal simulated controls are never intended to entirely replace liquid quality control, and the manufacturer recommends analyzing liquid controls with each shipment of cartridges, new lots of cartridges, whenever cartridges experience a temperature shift >8°C, or as required by the laboratory. Temperature is monitored continuously during each test, but a temperature verification cartridge is recommended at least annually.

The information about the test system and the function of the internal control processes can now be processed through a risk assessment. Risk assessment is best started by mapping the testing process to look for weaknesses and steps that could lead to error. Follow the sample from order to specimen collection, analysis, and reporting of results. Areas of focus should include the sample, the reagents, the operator, the analyzer, and the environment. Examine those hazards of greatest risk first including errors that occur frequently or lead to greater severity.

For compliance with federal and state regulations, testing should only be conducted based on a physician order. With POCT, operators can simply pick up the device and perform a test. So, operators must be trained to only conduct a blood gas or electrolyte test with this system based on an existing physician order. This should become an element of the operator training program. With appropriate training and demonstration of ongoing operator competency, the laboratory can conclude that risk of this error is reduced to a clinically acceptable level.

Blood gas samples should be collected anaerobically in electrolyte balanced heparin. Inappropriate collection or use of the wrong specimen additive can affect blood gas and ionized calcium results. Operators should thus be trained to utilize the appropriate sample and collection technique. Failure to adequately mix or overmixing the sample can further lead to clots or hemolysis of the sample. Whole blood samples continue to metabolize after collection, so prompt analysis, no more than 15 – 30 minutes after collection, is important. These are additional elements that should be added to the operator training and competency program to reduce risk of these errors.

Operator technique can impact POCT results, so the effect of operator technique is critical to assessing risk with POCT. Operator lock-out features on POCT devices require a personal identification number to unlock the device and perform patient testing. This feature ensures that only those trained and competent operators are conducting testing. Adding too much or too little sample can affect test results by flooding the cartridge or contaminating the connector pins, and insufficient sample failing to adequately contact the sensors in the cartridge. This analyzer has volume detection and will not allow overfilling or start a test until an adequate amount of sample has been added. The analyzer also automates all steps of the testing process, preventing incorrect timing, misinterpretation, or other procedural steps common for POCT. The analyzer also detects the expiration date of the cartridge through barcoding, preventing use of expired reagents. Documentation of results into the patient's medical record presents an

additional step for operators, so there is a risk of manual test results not being documented. The test system wireless connectivity and data management system ensure documentation of results without need for operator intervention or requiring additional operator actions. POCT devices can transmit nosocomial infections between patients, so cleaning and disinfection between patients is important. Training and reminders for staff on proper cleaning will effectively reduce risk of this error.

The cartridges contain the chemistry and detection sensors of the test system. Exposure of cartridges to temperatures outside of manufacturer specifications during shipping and lot-to-lot variation can affect test results. Analysis of liquid quality control upon receipt of new shipments and lots of cartridges can prove the viability of the cartridge prior to use for patient samples. However, cartridges can also degrade during storage, so temperature monitoring of storage conditions is required to ensure continued viability through the life of the cartridges. Temperature monitoring of liquid control sample storage is also important to ensure control viability. Periodic analysis of liquid quality control will further ensure cartridge and control stability. At what frequency should control samples be analyzed? The manufacturer recommends testing liquid control samples upon receipt of each shipment, with new lots of cartridges, and periodically to verify cartridge stability during storage. To determine the frequency of liquid control testing during storage, laboratories can perform side-by-side testing of daily liquid controls with internal control processes to document shelf stability for a period of several weeks. Once stability is documented for several weeks, the laboratory will have data to decrease the frequency of liquid control to every few days, and eventually weekly or monthly, depending on the life-span of cartridges after receipt.

Temperature and humidity can also affect the analyzer during analysis. The analyzer automatically detects environmental conditions which will impact analysis and warn the operator. The analyzer does not require water, works on battery power and internally detects the electrical circuitry and sensor connector pins. So, these risks are not a consideration with this device.

Once the testing process has been mapped, hazards recognized and control processes identified, the third step of the risk management process is summarizing the quality control plan. The quality control plan summarizes all of the hazards recognized during the risk assessment and the error mitigations selected, both those internal control processes from the manufacturer and the actions from the laboratory. The laboratory assesses whether the mitigations reduce risk to a clinically acceptable level. If risk is not reduced to an acceptable level, then the laboratory must take additional mitigation steps to control the risk. Such actions may include additional controls, maintenance, training or other actions.

The final step of the risk management process is implementing the quality control plan and monitoring the effectiveness of the plan. Benchmarking of the laboratory's quality can prove the effectiveness of the plan. Benchmarks for this blood gas and electrolyte analyzer could include trends in quality control, internal controls as well as liquid quality controls, analyzer error codes, physician complaints, or any other unexpected trends. When errors do arise, the laboratory should troubleshoot to determine the source, correct the process and reassess risk in light of the new information, modifying the quality control plan as required. This creates a continuous quality control process for the laboratory and this device.

Table 1 Example Risk Assessment: Blood Gas and Electrolyte POCT Analyzer

Example risk assessment for a generic unit-use POCT blood gas and electrolyte analyzer considering risks from samples, operator, reagents, device and the environment on the testing process

Hazard	Manufacturer Control Process	Laboratory Action	Risk Clinically Acceptable?
Physician order		Operator training	Yes
Anaerobic collection for blood gases		Operator training	Yes
Incorrect tube additive		Operator training	Yes
Clots, hemolysis (undermixing or over-mixing)	Clot and bubble detection	Operator training	Yes
Delays in analysis		Operator training	Yes
Operators trained/competent	Operator lock-out		Yes
Over-filling or under-filling	Sample detection		Yes
Incorrect operator procedure	Automated test analysis		Yes
Use of expired reagents	Expiration date bar-coded in cartridge		Yes
Failure to document results	Wireless connectivity		Yes
Forgetting to clean device		Operator training	Yes
Exposure during cartridge shipment		Analyze liquid quality controls	Yes
Lot-to-lot variability		Analyze liquid quality controls	Yes
Cartridge degradation during storage		Monitor storage conditions Analyze liquid quality controls	Yes
Device failure – electrical, sensor, computational	Internal checks and internal QC	Monitor error codes	Yes
Environment temperature and humidity	Continuously monitored		Yes

CONCLUSIONS

The US Centers for Medicare and Medicaid Services (CMS) recently implemented new Clinical and Laboratory Improvement Amendments interpretive guidelines in January 2014 (8). Risk management principles have been incorporated into the new interpretive guidelines in the form of Individualized Quality Control Plans (IQCP). CMS will begin inspecting for laboratory IQCPs beginning in 2016. At that time, laboratories will have two quality control options: 1) perform two levels of liquid quality control each day of testing or 2) develop an IQCP in order to reduce the frequency of liquid quality control. The laboratory cannot reduce frequency below manufacturer recommendations, and the laboratory must perform liquid quality control at some frequency (i.e., performing no liquid quality control is not an option.). Although IQCP will initially only apply to CLIA moderate complexity devices, any laboratory will benefit from mapping their processes and assessing weaknesses in their tests.

An IQCP provides several benefits for laboratories. Since the chemistry of the test reaction is in the unit-use test cartridge, facilities with dozens of the same device can select a subset of devices and rotate the analysis of liquid controls, since all devices share the same lot and supply of unit-use cartridges. For laboratory-developed tests, the laboratory can optimize the balance of liquid controls with manufacturer internal control processes. Most importantly, by developing an IQCP the laboratory will embrace industrial risk management principles and learn how to better detect and control risks with their test systems.

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Virtual support of a point of care testing network

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INTRODUCTION

Point of Care Testing (PoCT) continues to grow in hospital and general practice (GP) markets. PoCT has the opportunity to improve turnaround times for test results, increase patient satisfaction, improve the doctor to patient relationship and ultimately improve health outcomes for those involved.[1] However, these outcomes cannot occur in isolation. PoCT requires a coordinated approach involving all stakeholders and must occur in collaboration with a clinical team and be part of an integrated and innovative model to succeed.[2]

The PoCT model needs to take into consideration the environment in which it is to be implemented. In most cases, PoCT operators will be nurses and the PoCT will be an additional role for them to undertake, so this extra task must be regarded as a valuable use of their already stretched time. Nurses will be supportive of PoCT if it improves the efficiency of their patient care activities and where the clinical benefit clearly demonstrates that not performing PoCT would be detrimental to their patients.[3] If PoCT is regarded as just shifting work from laboratory to nursing staff, significant resistance will occur. This highlights the importance of appropriately assessing the clinical need for PoCT before it is implemented.

The model of centralised laboratory testing does not always meet the needs of health services in rural and remote areas and PoCT is regarded as a potential approach to overcoming this inequity.[4] For PoCT to be successfully implemented across all health sectors, information and communications technology (ICT) must play a vital role in supporting a PoCT service, particularly for widely dispersed networks covering rural and remote areas.

Hospital staff often face a resource and time poor environment with patient care justifiably requiring most if not all of their time. The issue of nurse staffing and funding continues to increase in the current economically constrained health environment.[5] These factors create time and funding barriers to staff attending training and education events. This is particularly evident in rural and remote sites whose training and education opportunities would require extensive travel to a bigger and more regional health centre. However the use of ICT to provide virtual support for PoCT can break down some of these traditional barriers, allowing PoCT to be used in an efficient and effective way.

A large randomised control trial of PoCT in general practice conducted in Australia in 2009 demonstrated that for most tests involved, central laboratory testing was more cost effective

than PoCT.[6] One of the leading factors that contributed to the excessive cost of PoCT was the considerable amount of resources devoted to face to face training PoCT users, many of whom were in remote locations. The utilisation of virtual support can significantly reduce this cost burden and provide a more cost effective and streamlined model for training of PoCT operators.

ICT can provide a virtual network to assist with training, education, certification, result management and decision support processes for PoCT operators within a cost effective network.

ONLINE TRAINING

Online information and training provides an effective method of ensuring all staff involved with PoCT are appropriately trained, regardless of their location. Training videos create moving step by step instructions for users. Accessible at any time, users are able to brush up on their skills and have visual and verbal prompts to follow when performing the tests themselves.

Hard copy methods should be made available for PoCT users to access when needing a refresher on the test they are performing. This also allows the users to become responsible for their instruments and teaches them to investigate their processes and methods in order to gather a greater understanding, before reaching for the phone to call for assistance.

Troubleshooting documentation including a list of common error codes should also be made available online. Users can interrogate these documents to ascertain where their process may be going wrong and what they can do to fix it.

VIDEOCONFERENCING

The use of videoconferencing facilities enables direct user training with visual and auditory influence. This system removes the traditional barrier of distance and ensures all PoCT operators can be comprehensively trained in the use of each instrument. User training and instrument troubleshooting can all be performed via the videoconferencing network, with the trainers able to see exactly what is happening with the instruments at the user end.

ONLINE CLINICAL EDUCATION

Clinical education traditionally shared at conference and face to face meetings can also be provided in a web based format accessible to staff from any location. Presentations can be recorded and loaded on to a central online education site for staff to view in their own time and from their own health facility. Innovative programs such as webinars and web conferencing can be an even more successful alternative, with users viewing the education live and being able to ask questions to the presenter while the presentation is occurring. These presentations can also be recorded and stored centrally for review.

ONLINE CERTIFICATION

Competency assessment is vital in maintaining the skills of all those participating in a PoCT network. Assessments should be performed on all aspects of the PoCT system including operation of the equipment, sample collection, performing quality control testing, troubleshooting and clinical knowledge.

Continuing Professional Development (CPD) points are able to be allocated to the assessment processes and completion certificates should be made available for users to keep their own records of competency assessment. Competencies should be repeated at least yearly to ensure user's skills are maintained.

ELECTRONIC RESULTS DATABASE

Patient PoCT results should be transferred to an electronic database for storage and review

at any time. Results should be made available to all health professionals involved in the patient's care and access should not be limited to strictly internally based networks. An electronic results database allows a quick and easy update of the patient's medical history without needing to source paper based notes which may be off site, thereby creating a more streamlined patient care environment.

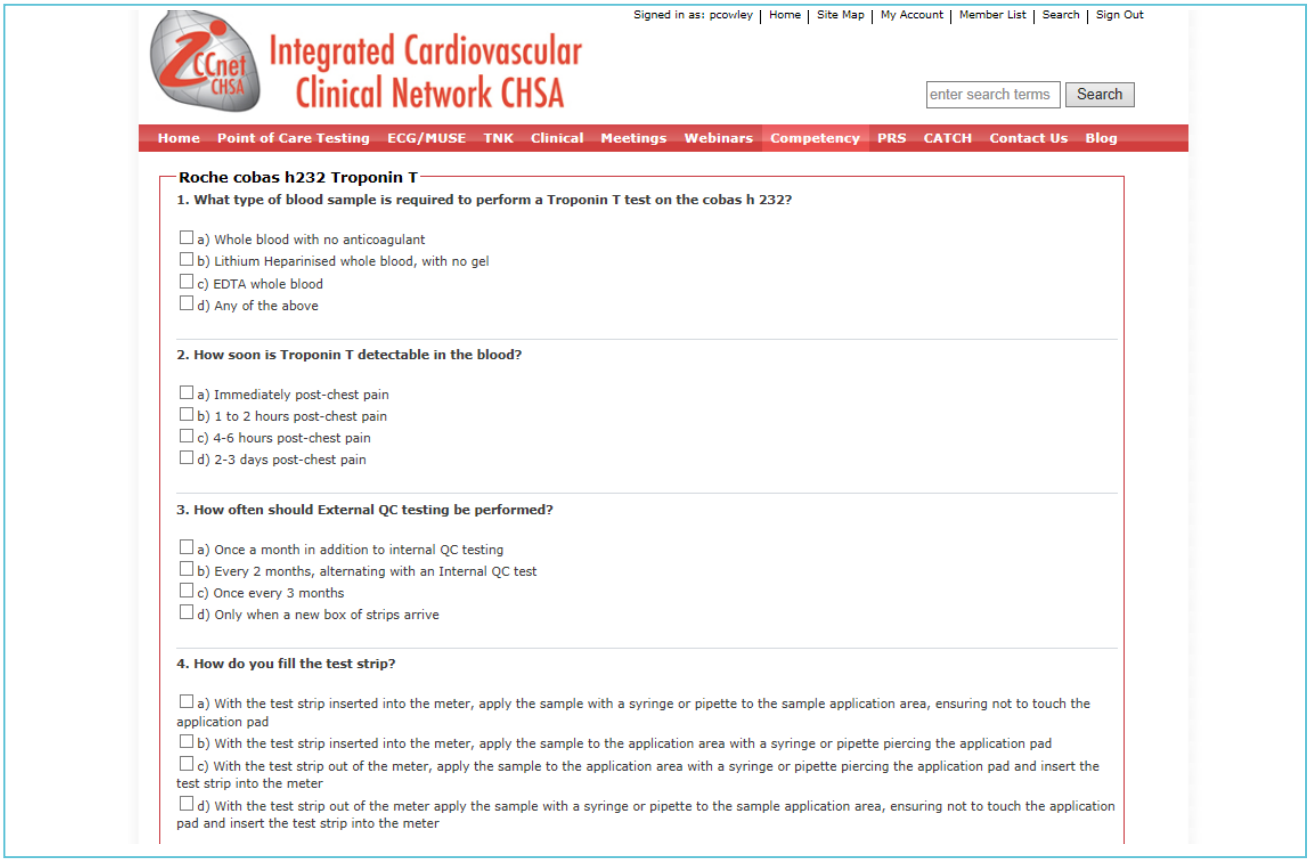
ONLINE QUALITY CONTROL MANAGEMENT

Quality Control (QC) results should also be transferred to an electronic database. Electronic QC systems allow for off site management of instrument performance, electronic monitoring of user compliance and enable early identification of instrument inaccuracy and off site troubleshooting. These systems should be made available to all PoCT users in the network to promote engagement of all users in the quality management process.

ONLINE PATIENT MANAGEMENT

Where possible, the use of online patient management systems should be accessible to aid health professionals in managing their patients. Medication and treatment management can incorporate evidence based guidelines to create an easy to follow protocol. These management systems are particularly useful for general practitioners who may not be exposed to these patient situations on a regular basis.

Figure 1 An online multiple choice competency assessment, with questions pertaining to all aspects of the PoCT device



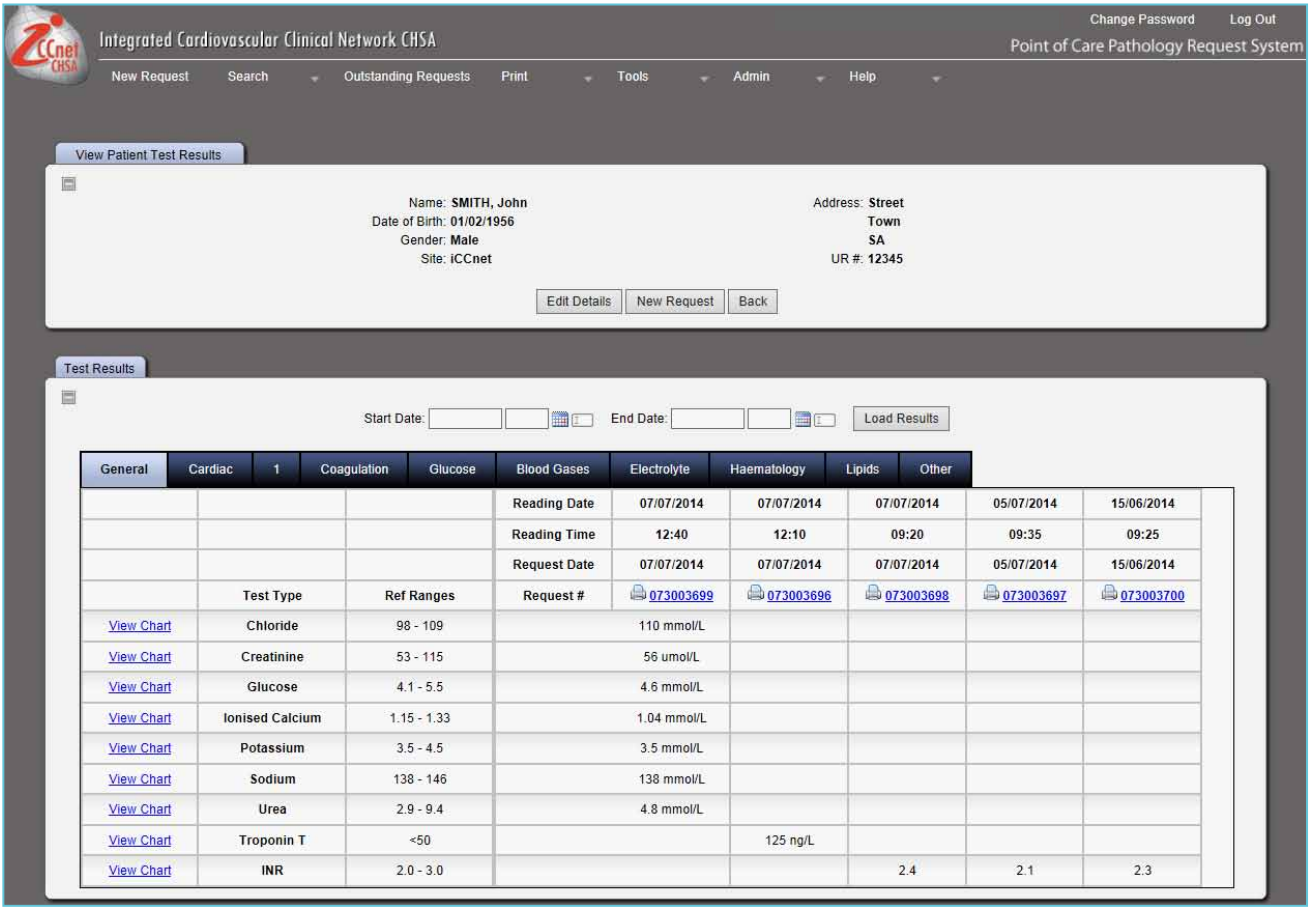
iCCnet

The Integrated Cardiovascular Clinical Network Country Health SA (iCCnet CHSA) was developed in 2001 to support general practitioners and nurses in rural areas in delivering first grade evidence based cardiac care. A key pillar of this evidence based cardiac care was the utilisation of rapid point-of-care testing for Troponin, which was successfully introduced to all 66 CHSA rural hospitals by 2008.[7] The PoCT service has since expanded to cover a wide spectrum of pathology tests, all embedded into a quality framework involving instrument quality control and assurance, competency assessment and regular education of all users.

With the area of South Australia covered by iCCnet stretching to almost 1 million square kilometres[2], implementation of a virtual support service has become critical to the success of the network. Online training, clinical education and certification are all provided by the iCCnet website. Videoconferencing is now employed as a major form of user training and quality control & patient result management is now achieved through an online database to complete the virtual network.

The iCCnet CHSA website contains all information pertinent to the testing process for PoCT users. Use of the website is free and users are approved by the iCCnet team. Online training including training videos and certification are provided through the iCCnet website for all instruments. Competency assessment is through multiple choice questionnaires randomly selected from a pool of test questions and a 100% pass rate is required (Figure 1). Certificates are

Figure 2 PoCT Pathology Database showing the PoCT patient record



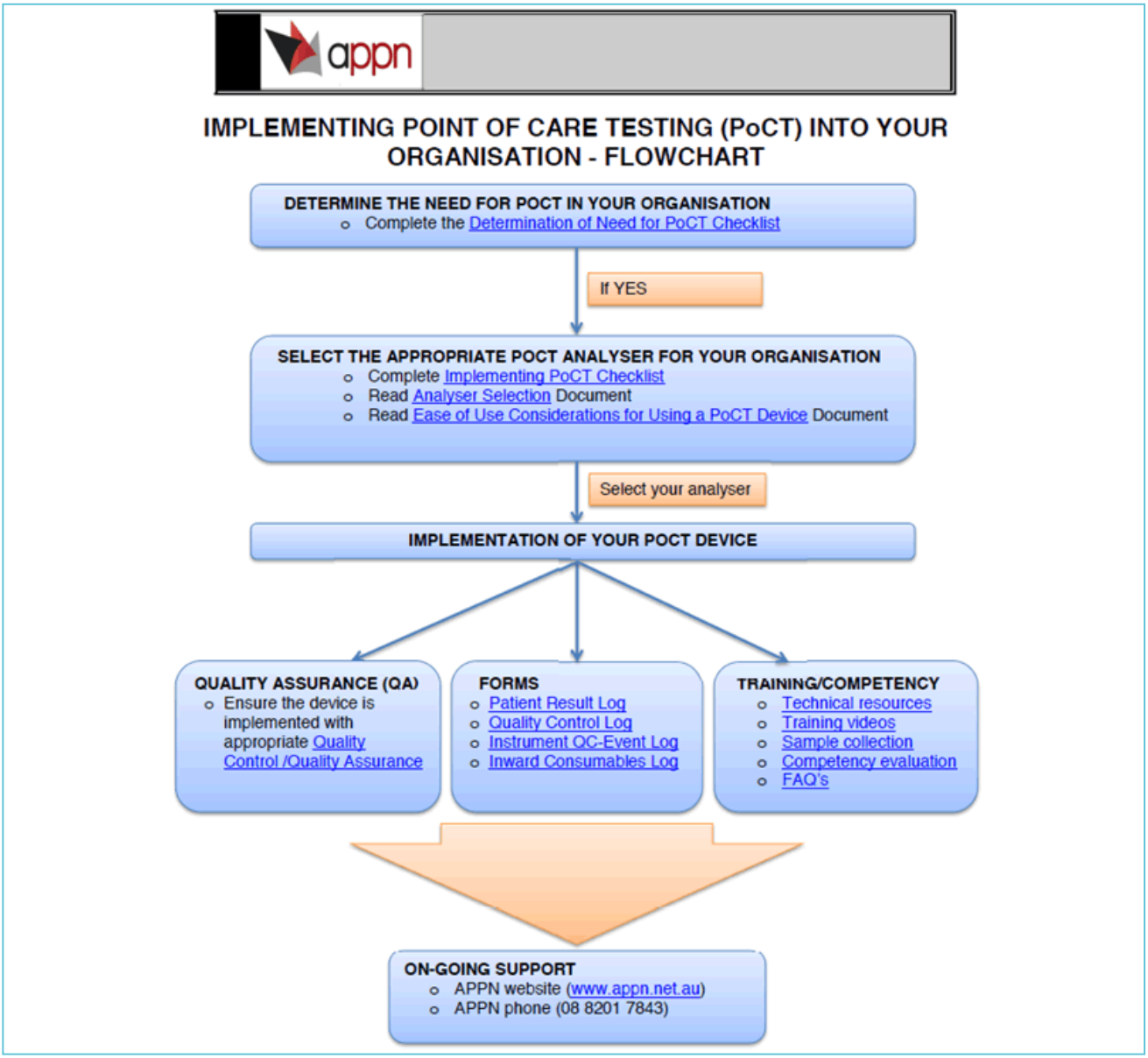
generated with a 12 month expiry, with users notified when their competency has lapsed.

The majority of user training is conducted via videoconferencing. The upgrade of videoconferencing facilities in all hospitals throughout South Australia (SA) in 2013 enabled clear images and high sound quality for all sites including those connecting from as far as 1400 kilometres away. These facilities provide a cost effective method for training PoCT users without the need for travel.

The upgrade of instrument software and tracking of instruments is all controlled over the iCCnet network, with two way communication from the network to the instruments.

The newest addition to the virtual iCCnet suite has been the PoCT Pathology Database for collection and storage of all patient PoCT results (Figure 2). Instruments from any manufacturer

Figure 3 APPN guidelines for implementing PoCT



are able to be connected to the Pathology Database, with 9 instrument types from 5 different manufacturers totalling 305 individual instruments currently connected.

Test results are transferred directly from the instrument to the database over a secure network and stored in the individual patient record. Pathology reports are generated for storage in hard copy notes or for emailing to doctors. PoCT consumables are able to be ordered through the system and an inventory log of all consumables on site is automatically updated with each order received and each test performed. Positive patient results are tracked daily and sent to system managers, allowing auditing of these patients to ensure protocols are followed and desired outcomes are met.

Quality Control results are also transferred directly into the system and compliance is monitored with emails automatically sent when a QC rule has been exceeded. Instrument serial numbers are tracked and out of range results are alerted allowing instrument specific troubleshooting. The system is available through a secure login on the internet, with any authorised health professional able to review PoCT results for all tests performed in Country SA hospitals.

APPN

The Australian Point of Care Practitioners Network (APPN) is a web based program funded by the Australian government to provide a comprehensive resource for all PoCT operators, including professional development.[8] The APPN provides clinical and technical information based on clinical disease appropriate for all health professionals.

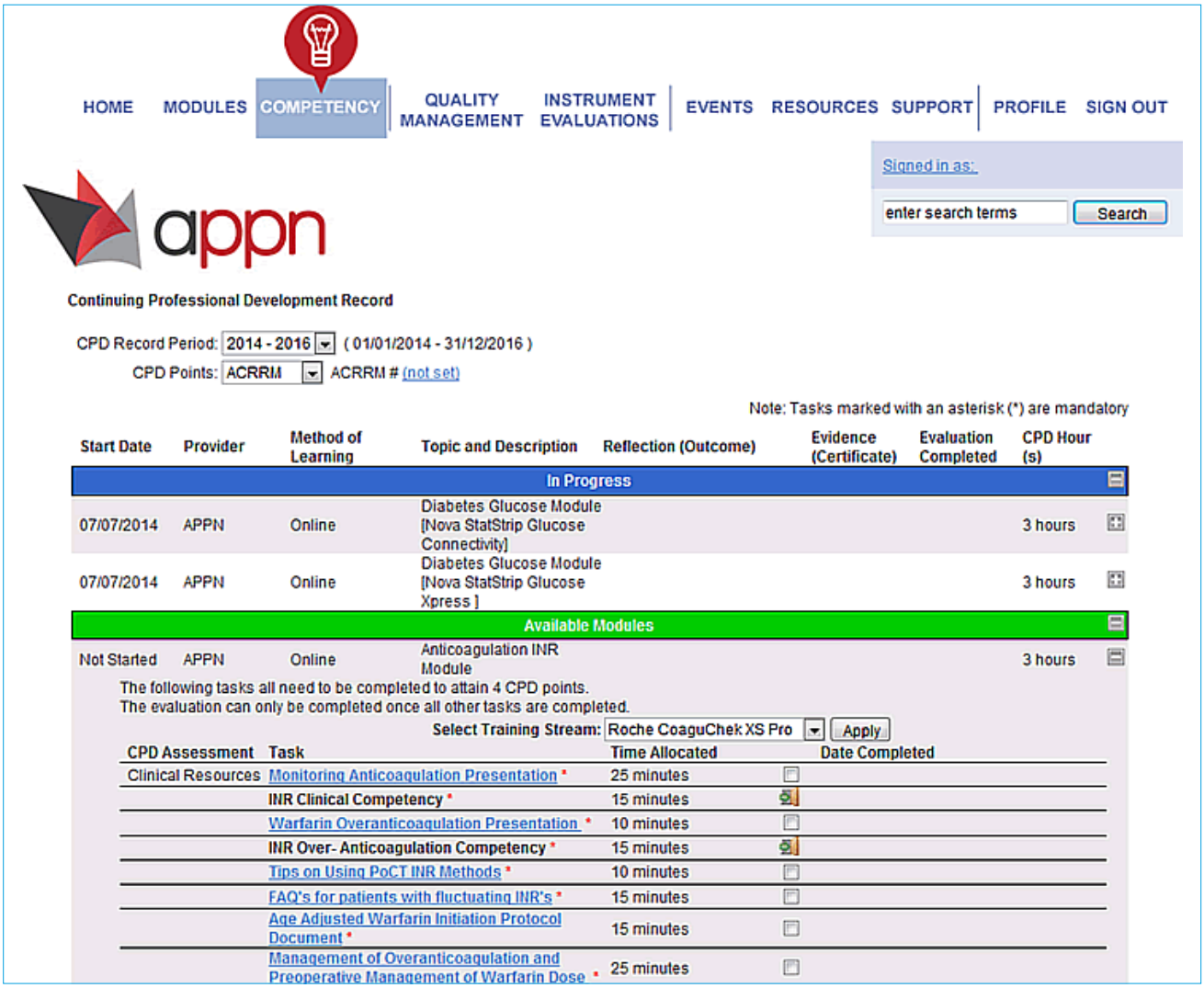
APPN registrants numbered almost 2000 in 2014, comprising both domestic and international members.[8] Online training, clinical education and certification are all provided through the APPN website including a personally controlled CPD record. The PoCT process is covered from start to finish, including guidelines for implementing PoCT in your practice (Figure 3). Quality controls are managed through the website and online patient management systems are being developed through the APPN service to complete the virtual network.

The APPN website contains education around all PoCT equipment available in Australia. This includes methods, troubleshooting and clinical information. Webinars are regularly broadcast on multiple topics requested by users. Competency assessment is available for all modules and CPD points are able to be self-allocated for nursing and medical professionals. An electronic CPD record is available for all users, containing completed modules, completion certificates and alerts for assessments needing refreshing (Figure 4). Results of the quality control performance of APPN PoCT operators receiving online training and PoCT operators receiving to face-to-face training suggests that web-based training is an adequate training method for PoCT users.[8]

An online Quality Management Program is also available for general practice on the APPN website. This system allows the management of QC results and consumables online. Results are entered on to the system by each practice and are flagged as a pass or fail. Results flagged as a fail must have a reason identified and a corrective action must be performed to enable the safe and accurate use of that instrument. Results are able to be entered on a generic practice login, ensuring all PoCT users are involved in the quality management process.

The APPN has designed an online protocol to guide the initiation and monitoring of warfarin treatment in patients with AF. The age adjusted warfarin initiation protocol was implanted as the background algorithm to determine

Figure 4 **A user's CPD Record, demonstrating available, in progress and completed APPN online modules**



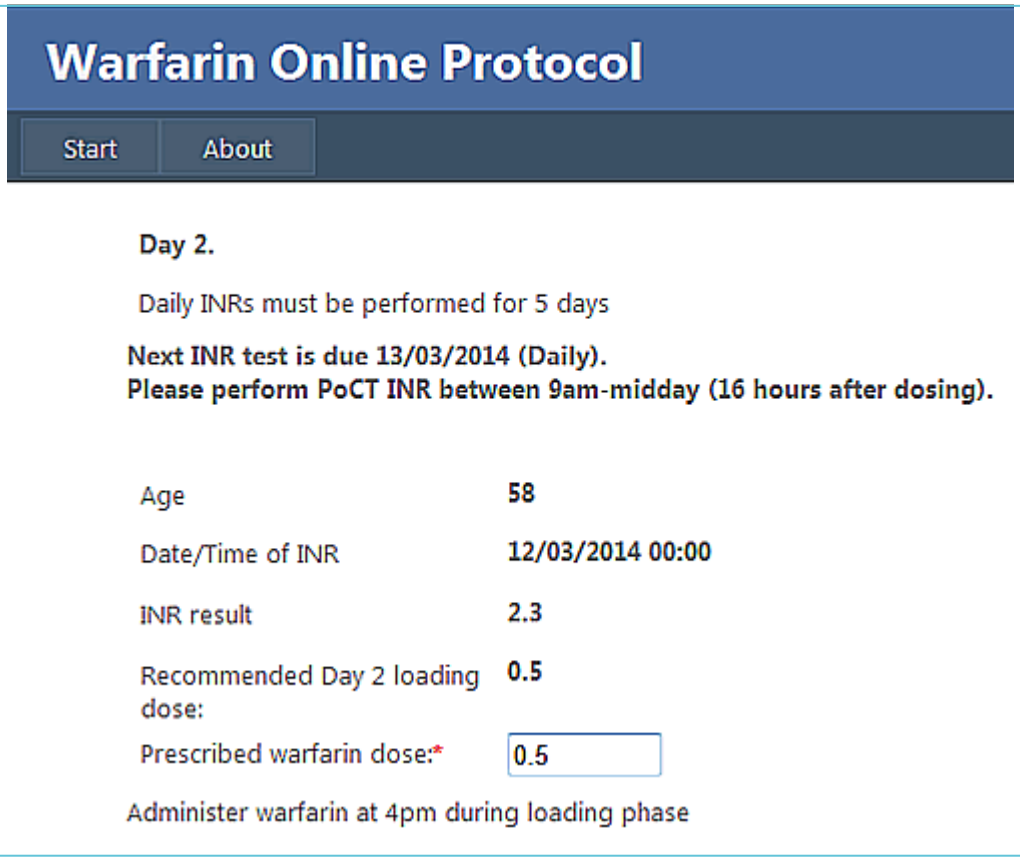
warfarin dosing and PoCT embedded as the clinical contributor (Figure 5).

Through input of date of birth and PoCT INR result, the background algorithm derives the recommended warfarin dose. The recommended follow up date is listed and a printable calendar showing warfarin dose and time of next INR test is displayed along with a graph plotting all INR results. The time in therapeutic range (TTR) is calculated for each patient and for the practice overall to determine effectiveness of treatment.

CONCLUSION

There is a need for better access to pathology testing in rural and remote Australia and this can only be provided by PoCT. To be provided in a cost effective manner, virtual support through information and communications technology must be employed. Such support can train and educate users, provide online databases to track and report patient and quality control results and incorporate online decision support to aid health professionals in treating their patients effectively.

Figure 5 The APPN warfarin online protocol for warfarin initiation



It holds the key to streamlining PoCT implementation and integrating it into clinical management of patients and professional development of operators.

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Recent advances in point-of-care diagnostics for cardiac markers

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Cardiac troponin, B-type natriuretic peptides, point-of-care testing, microfluidics

ABSTRACT

National and international cardiology guidelines have recommended a 1-hour turnaround time for reporting results of cardiac troponin to emergency department personnel, measured from the time of blood collection to reporting. Use of point-of-care testing (POCT) can reduce turnaround times for cardiac markers, but current devices are not as precise or sensitive as central laboratory assays. The gap is growing as manufacturers of mainframe immunoassay instruments have or will release troponin assays that are even higher than those currently available. These assays have analytical sensitivity that enables detection of nearly 100% of all healthy subjects which is not possible for current POCT assays. Use of high sensitivity troponin results in a lower value for the 99th percentile of a healthy population. Clinically, this enables for the detection of more cases of myocardial injury. In order to compete analytically, next generation POCT assays will to make technologic advancements, such as the use of microfluidic to better control sample delivery, nanoparticles or nanotubes to increase the surface-to-volume ratios for analytes and antibodies, and novel detection schemes such as chemiluminescence and electrochemical detectors to enhance analytical sensitivity. Multi-marker analysis using POCT is also on the horizon for tests that complement cardiac troponin.

BACKGROUND AND CURRENT POCT DEVICES

The National Academy of Clinical Biochemistry has recommended a 1-hour turnaround (TAT) time for reporting of cardiac troponin (cTn) results, beginning with sample collection and ending with reporting (1). When troponin testing is conducted from the central laboratory, this goal is a challenge for most laboratories to meet. The sample must be labeled (1-2 min), put into an appropriate transportation container (2-5 min), sent to the laboratory (5-10 min), accessioned (5-10 min), centrifuged (10 min), delivered to the testing laboratory (1-5 min), loaded (1-5 min) and tested (20 min), results reviewed where appropriate (0-5 min), and finally released to the caregivers. Due to the difficulty of meeting this aggressive turnaround time goals, the in vitro diagnostics industry has been challenged to produce point-of-care testing (POCT) devices that have equivalent analytical sensitivity for measuring cardiac troponin.

POCT for troponin has been available for nearly 20 years. Among the earliest commercial point-of-care devices were qualitative lateral flow assays (e.g., Spectral Diagnostics for cardiac troponin I and Roche for troponin T). Whole blood samples containing the targeted analyte flow through a filter which separates plasma from erythrocytes. It is also mixed with a detecting antibody. This combination flows past an immobilized capture zone containing a second troponin antibody. The presence of troponin in the sample causes a line to be visualized. Shortly after the creation of qualitative assays, quantitative assays were constructed using small optical readers (Roche Cardiac Reader for troponin T and Alere Triage for troponin I). The iSTAT (Abbott) was among the first point-of-care device to make use of microfluidics to navigate sample through the various zones of the device. There have also been bench-level whole-blood analyzers that can be used for near-patient testing such as the Stratus CS (Siemens), AQT-90 (Radiometer), and Fastpath (Mitsubushi). These instruments have higher analytical sensitivity and precision than the hand-held POCT devices, and are equal to those obtained from the central laboratory.

There have been several studies that have documented the reduction of turnaround times when point-of-care testing has been implemented in the emergency department versus the central laboratory (Table 1) (2-6). With POCT, each of these studies demonstrated compliance with the 1-hour guideline. In contrast, none of the tests done in the corresponding central laboratory met the guideline. While many laboratories today are able to meet 1-h turnaround times, if 30 minutes is necessary, as desired by some ED physicians, then POCT becomes the only option.

"HIGH SENSITIVITY" TROPONIN ASSAYS FROM THE CENTRAL LABORATORY

The performance of central laboratory analyzers for troponin continues to undergo improvements with regards to analytical sensitivity and precision. Figure 1 shows that a significant amount of myocardial necrosis was required for detection of troponin using troponin assays that were first released into the market. Current generation troponin assays have about 10 fold higher analytical sensitivity and can detect the initial onset of myocardial infarction earlier. High-sensitivity troponin assays can detect normal levels and the earliest increases of troponin after myocardial infarction, micro necrosis, and possibly reversible ischemia as well.

There are several strategies that manufacturers have used to achieve the sensitivities needed to qualify as high sensitivity assays. These include use of longer incubation times, larger sample volumes, use of more than 2 antibodies for capture/detection, and use of chimeric antibodies

to improve avidity towards the target protein. In terms of using longer incubation times, there to how long the analysis time can be practically extended. Increasing the overall analysis time from 20 to e.g. 40 minutes, is self-defeating, as the objective of POCT testing is reducing TATs. Increased sensitivity can be achieved by using additional antibodies and capturing troponin fragments where the epitope towards the primary antibodies have been lost. Troponin is known to degrade at both the C and N-terminus (7). Figure 2 illustrates the use of an additional antibody enables better detection of degraded fragments. Chimeric and humanized antibodies are formed by recombinant DNA techniques and are a mix of human and non-human antibody sequences. Use of these antibodies minimizes interference when human anti-mouse antibodies (HAMA) are present (8).

GAP BETWEEN CURRENT POCT AND CENTRAL LAB ASSAYS FOR TROPONIN

Cardiac troponin is used for diagnosis of acute myocardial infarction (AMI) and risk stratification for future adverse cardiac events. Figure 3 illustrates the difference in analytical sensitivity between a POCT assay and the central laboratory (9). Singh et al. performed a direct comparison of a POCT assay versus the central lab (10). Out of 206 samples, there were 32 samples with discordant results. The majority of these discrepancies (82%) had a positive result on the central lab and negative result on the POCT assay. Improvements in the analytical sensitivity of troponin assays enable an early detection of AMI (11).

Improved sensitivity also allows the use of low cutoff concentration which improves the utility of troponin for risk stratification. Many studies have shown that patients who present with chest pain and have a minor increase in cardiac troponin have a higher incidence of adverse events (death, AMI) at 30 days and 1 year. James et al. compared the performance of a POCT cTnI assay against a central lab cTnT assay (12). The odds ratio for 30-day death or MI was 1.64 (95% CI: 1.31-2.06) for the POCT assay and 4.29 (CI: 3.02-6.09) for the central lab assay. These reports suggest that there will be a compromise in clinical performance when POCT assays are used in lieu of the central laboratory. To overcome these limitations, some clinical laboratories send samples tested by POCT devices to the central laboratory for repeat testing. This can lead to confusion by the physicians, while increasing the cost for testing. An alternative approach is to develop POCT assays that are as sensitive as the central laboratory.

GOALS FOR NEXT GENERATION POCT ASSAYS

The definition of myocardial infarction underwent a dramatic change in 1999 with the adoption of troponin as the preferred biomarker (13), replacing the WHO definitions (14). This definition has undergone refinements. The Third Redefinition of Myocardial Infarction affirmed the concept that the cutoff concentration is established at the 99th percentile of a healthy population with assay imprecision of 10% or less (15). For the best assays currently available in the U.S., this equates to a cutoff of between 25-40 ng/L. Current POCT devices cannot meet this sensitivity. Near-patient instruments for troponin are able to match the sensitivity limit of these existing assays.

Next-generation high sensitivity (hs) troponin assays have been developed and are available outside the U.S. and from CLIA-certified reference laboratories as a lab developed test. These assays have a 99th percentile cutoff of about 10 ng/L and a limit of detection of <1 ng/L. High-sensitivity assays are able to detect troponin in the majority of healthy subjects (16). The

analytical sensitivity of these assays may now be sufficient to meet current and future clinical needs, and further improvements in analytic sensitivity will be unnecessary. This is especially true because there is no clinical value for detecting troponin concentrations that are below the normal range. For POCT, these specifications for hs-cTn central laboratory assays are the goals for next-generation devices.

APPROACHES TOWARDS NOVEL POCT ASSAYS

Commercial interest in novel POCT assays stems from the fact that there are large numbers of patients who present to an emergency department each day with chest pain requiring troponin testing, and the existence of international guidelines that recommend a rapid turnaround time for reporting results. Unfortunately, developing POCT assays that meet these needs has been challenging. Described below are approaches that have been taken to improve POCT troponin testing (names of companies developing these prototype assays have been purposely omitted).

Microfluidics

Passive lateral flow technology is an inadequate means to deliver sample to the measurement zones, as this has been associated with high analytical imprecision. Differences in the viscosity of real blood samples due to variances in hemoglobin and protein content can limit precision, which impacts on analytical sensitivity. Next-generation POCT assays will most likely make use of microfluidic technology. Through the use of built-in pumps and valves, this advance enables precise movement of fluids and reagents from the point of sample application to the measurement zones within a POCT device. Washing of unbound antibodies or reagents can also be better controlled. Improved quality control schemes is possible including the testing of specific analytes that can be incorporated with each device instead of a simple check of fluid flow as is the case with lateral flow POCT. Due to its increased complexity however, the costs of microfluidic devices are higher than later flow technology.

Increased surface area-to-volume ratio for antibody-antigen reactions

All troponin assays are based on the reaction of the analyte with antibodies. Within the finite limits of the detection zone, the analytical sensitivity is a direct function of the ability of the assay to capture as much as the antigen as possible. The manufacturing of nanoparticles and nanotubes has become consistent and reliable. Their use in lieu of micro particles can greatly increase the surface-to-volume ratio over the micro particles used in central laboratory immunoassays. This enables the immobilization of a higher density of capture antibodies thereby retaining and detecting as much of the target analyte as possible.

Novel detection schemes

Visual detection of labeled gold micro particles has inherent sensitivity limitations. For the same number of troponin molecules captures, the use of other detection schemes can substantially increase the analytical sensitivity of troponin assays. Among the novel signal technologies used include fluorescence, chemiluminescence, and electrochemical detection. This migration to more sensitive technologies is consistent with the advancement made in the central laboratory where spectrophotometric measurements of immunoassays labeled with enzymes have given way towards chemiluminescence and electrochemistry. The challenge is to make miniaturize detectors so that they can be applicable to POCT. Relative to visual detection, these advanced detectors can increase the assay sensitivity 10-100 fold.

Connectivity advances

A disadvantage of current POCT assays is connectivity between measuring device and the patient's medical records. The documentation and dissemination of test results is of critical importance to the effective delivery of testing. Central laboratory and bench-top satellite laboratory instruments can be directly interfaced to a laboratory or hospital information system. Hand-held devices require wireless transmission of data to the appropriate portals. The worldwide advance in telecommunications will allow caregivers direct access to secure medical information through pagers and smart phones. This will be a requirement for next-generation POCT devices.

Regulatory issues

In the U.S., all clinical assays must be approved by the Food and Drug Administration prior to routine clinical use or be validated as a "Laboratory Developed Test." The FDA lists levels of complexity and requirements for utilization of tests. All central laboratory and POCT troponin assays are currently listed as "moderately complex tests." This requires a certain degree of training and supervision of the testing personnel. "Waived test have less stringent requirements for testing personnel. Manufacturers of next-generation POCT troponin assays should consider seeking waived status for their devices. This will accelerate adoption of POCT in the ED. There is one POCT assay for BNP that is FDA cleared as a waived test. The history of its submission and approval could be a model for getting a next-generation POCT troponin assay cleared as a waived test.

On-vitro analysis

A unique concept for POCT could be "*on-vitro"* analysis. While *in vitro* refers to the testing environment outside the body and *in vivo* refers to studies within the body, the term "*on vitro*" could refer to a term whereby testing is conducted outside the body, but the device is placed on the skin of the patient. Blood is automatically sampled and tested within the device on demand or at regular intervals while worn. There are diagnostic companies on vitro devices for painless collection of blood, particularly for neonates. Samples contained within the device could be directed by microfluidics to test areas. *On vitro* diagnostic tests may be convenient and ideal for cardiac markers as serial testing is required for accurate diagnosis and rule out.

SUMMARY

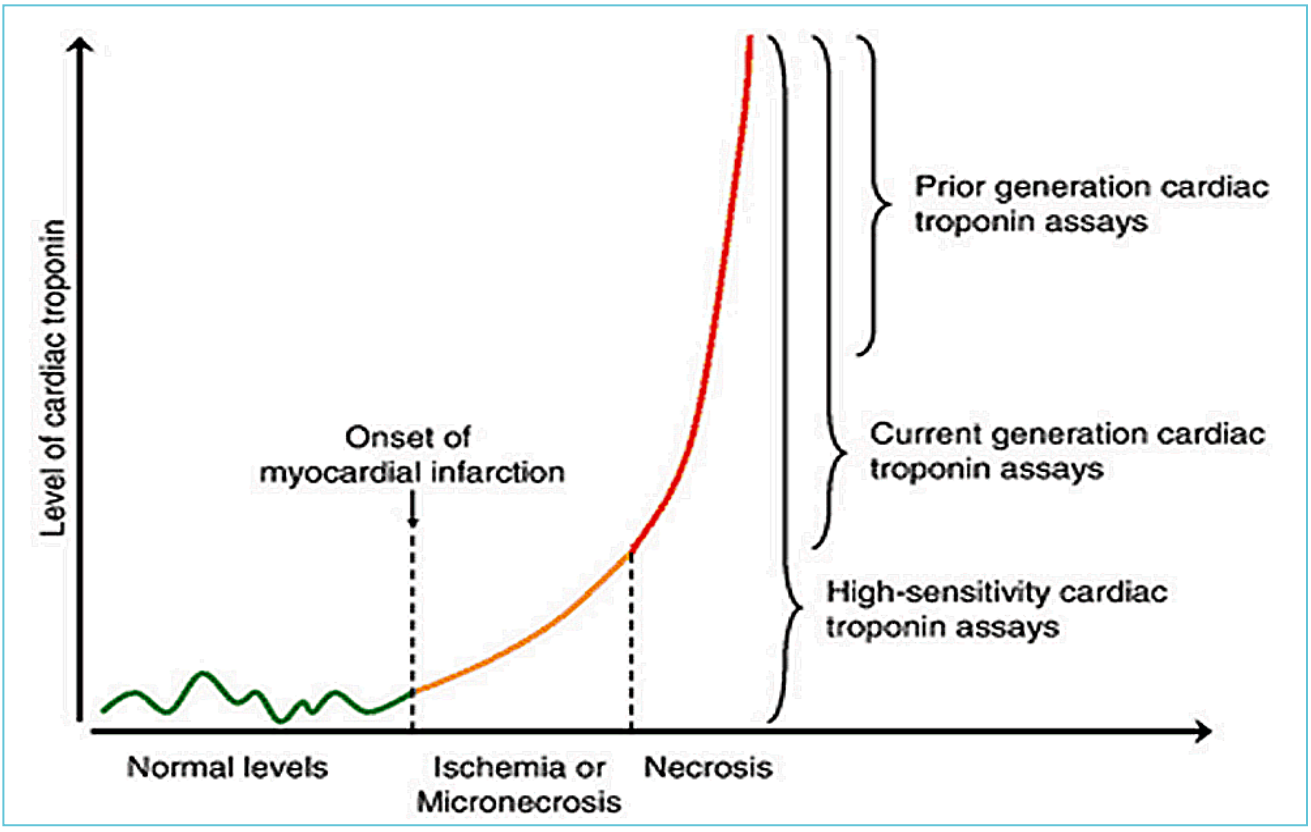
The analytical sensitivity gap between central laboratory testing platforms and POCT assays for cardiac troponin is significant and has hindered the adoption of POCT for many hospitals. Although not discussed, there may also be a need for POCT platforms that can undergo multi-marker analysis. While troponin is the main analyte for AMI diagnosis, B-type natriuretic peptide (BNP) and NT-proBNP have shown to be useful for short-term risk stratification. There are also other biomarkers that can be used for the early rule out of AMI such as competing (17).

High sensitivity troponin might also be useful as a risk stratification marker in primary care, i.e., for patients who are asymptomatic (18). This is based on observations that increased troponin is associated with high risk for adverse cardiac outcomes in the absence of acute coronary syndromes (19). If this becomes adopted as part of routine medical care for high risk patients, then POCT for hs-cTn may be useful and convenient when tested in physician offices and clinics. Therapeutic measures such as the administration of statins, beta blockers or an angiotensin converting enzyme inhibitor can be prescribed before the patient leaves the office.

Table 1 Published reports on the reduction of turnaround times using point-of-care testing

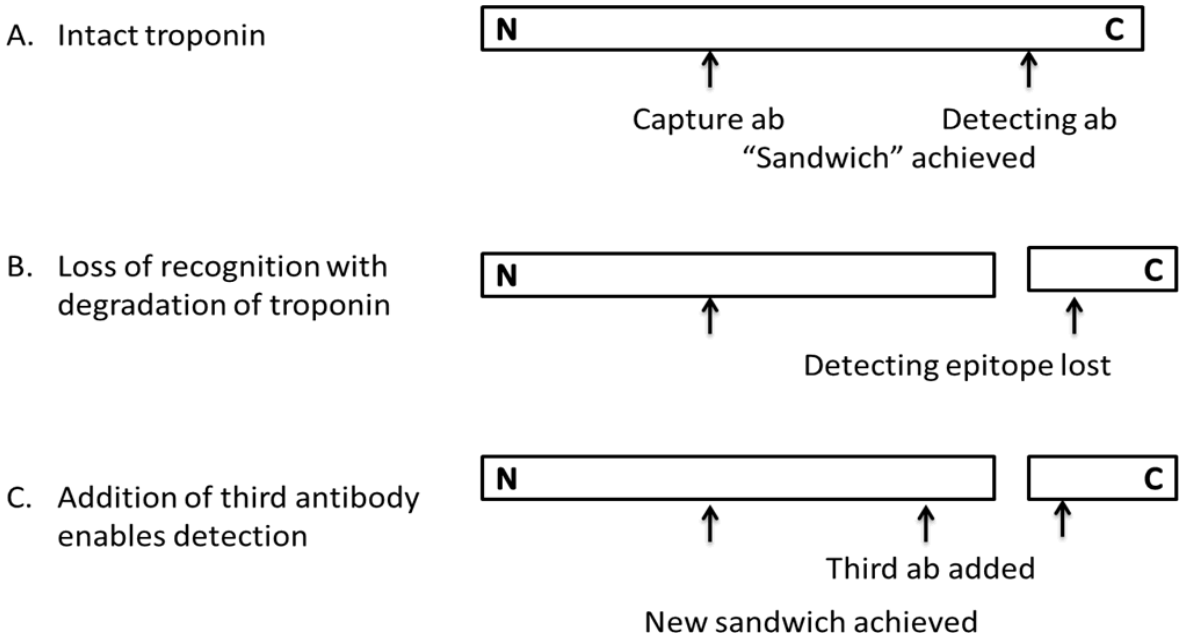
Study	POCT	Assay	Central lab	Δ, ↓
McCord, et al. 2001	24	Triage	71	66%
Caragher et al., 2002	38	Stratus CS	87	56%
Lee-Lewandrowski et al. 2003	17	Spectral	110	85%
Collinson et al. 2004	20	Triage	79	75%
Singer et al. 2005	15	Stratus CS	83	85%
Mean	23		89	79%

Figure 1 Analytical sensitivity improvements with different generations of troponin assays



Used with permission from Hochholzer et al. Am Heart J 2010;160:583-94

Figure 2 Increased sensitivity with the addition of a third antibody to the assay

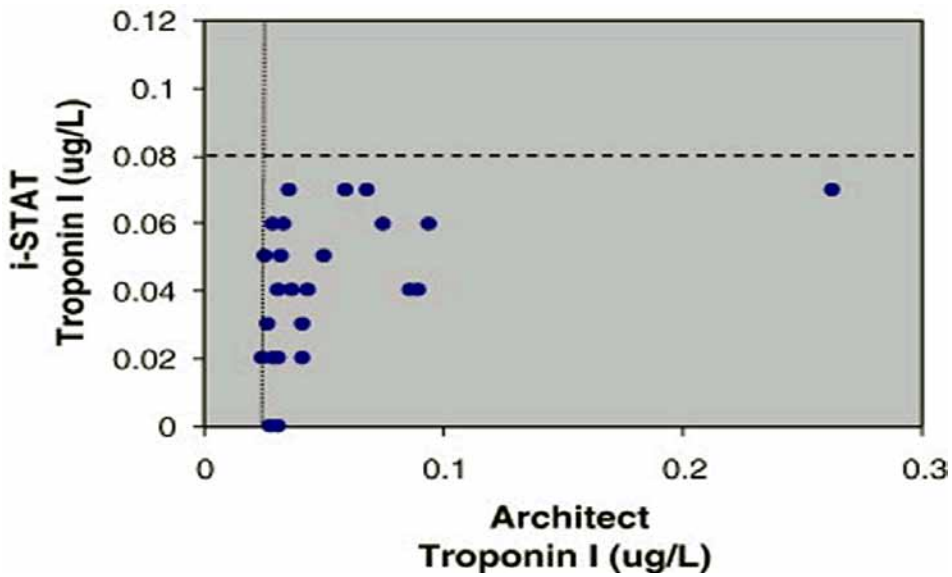


A. Detection of an intact troponin molecule with a single capture and detection antibody using a two-site "sandwich" immunoassay.

B. Degradation of troponin at the C-terminus. The epitopes of the capture and detecting antibodies are now on different fragments therefore neither are detected.

C. Use of a third antibody enables detection of the larger fragment of troponin.

Figure 3 Discordant results on the iSTAT with positive results on the Architect



Cutoff concentration was 0.08 and 0.025 mg/L, respectively.

Used with permission from Singh et al. Clin Chim Acta 2009;403:259-60.

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Glucose meter use in the intensive care unit: much ado about something

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ABSTRACT

Glucose meters are a fast and convenient way to measure circulating blood glucose. Like many technologies in healthcare, the use of glucose meters within the hospital has evolved significantly over the last few decades. This change has been driven predominantly by changes in the approach to glycemic control for critically ill patients. Both glycemic control in the intensive care unit (ICU), and use of glucose meters to manage insulin dosing during glycemic control, are likely to remain controversial topics in the years to come. This review will elaborate on the evidence for and against use of glucose meters in the ICU to monitor glucose concentrations during glycemic control, and provide some tips for point of care programs on how to evaluate glucose monitors for this purpose.

INTRODUCTION

Glucose meters have been used in the hospital setting for decades. Traditionally glucose meters were used in the hospital to dose subcutaneous insulin for patients with diabetes when they were hospitalized. As even well-controlled diabetic patients will have their insulin needs, diet and caloric requirements change during periods of acute illness; glucose must be measured frequently (four or more times per day) before meals and/or insulin dosing in the hospital. Although most hospital laboratories offer a measurement of serum or plasma glucose, hospitals and healthcare systems find it both convenient and efficient to measure capillary whole blood glucose at the bedside in order to expedite insulin dosing. This can help insure that glucose values are taken before (rather than after) meals are consumed, as it is the preprandial blood sugar value that is most often used to dose insulin.

In 2001 Dr. Van den Berghe and colleagues changed the landscape of glucose control in the hospital by studying the impact of tight glycemic control (maintaining blood glucose between 80-110 mg/dL) among critically ill patients (both diabetic and non-diabetic) after cardiovascular surgery. Dr. Van den Berghe's original study sought to determine whether closely controlling glucose levels in patients in a surgical intensive care unit (ICU) would improve patient outcome. In the study 1500 patients were divided into two groups: one control group that received what was conventional treatment of hyperglycemia in the ICU at that time (subcutaneous or intravenous insulin to keep glucose levels less than 200 mg/dL), and an experimental group that received intravenous insulin to keep blood glucose at relatively normal levels of 80-110 mg/dL. The experimental group that received intravenous insulin to keep blood glucose relatively normal had much better health outcomes than the control group (mortality decreased 34%, renal failure 41%, bloodstream infections 46%)[1]. The outcomes were startling to critical care experts, and almost overnight changed the standard of care in critical care medicine from a relaxed attitude towards hyperglycemia in the ICU to vigilant glucose monitoring and insulin treatment to maintain normal or near-normal blood glucose levels.

Subsequent studies found that depending upon the patient population (medical vs. surgical ICU), ICU nutrition practices, and protocols to dose insulin and monitor glucose; intensive glycemic control was of either benefit in only some ICU patients or not beneficial at all[2-4]. Finally, in 2011 a multi-center trial called NICE-SUGAR was performed to determine what level of glycemic control was optimal in the ICU setting. Unlike the preliminary studies done by Dr. Van den Berghe, NICE-SUGAR did not compare "conventional treatment" to more rigorous management of glycemic control; as by that time some active management of glucose levels in the ICU was standard of care. Rather, NICE-SUGAR compared two different glucose management strategies—one aimed at controlling glucose levels among critically ill patients to near-normal levels (similar to the Van den Berghe strategy) and one that aimed for slightly higher (140-180 mg/dL) glucose levels. NICE-SUGAR, performed in over 40 medical centers, found that patients assigned to the higher (< 180 mg/dL) glucose target had significantly better health outcomes than those whose glucose target was near-normal (81-108 mg/dL)[5].

Among the reasons why more moderate glucose targets may be beneficial to critically ill patients, rates of hypoglycemia are most commonly cited. All studies of intensive glucose control in the ICU, including the original studies by Dr. Van den Berghe, found that rates of hypoglycemia are higher among patients whose glucose levels are controlled actively with intravenous insulin. In

fact, studies have shown that intravenous insulin therapy increases the rate of hypoglycemia among ICU patients on average 5-fold[4]. This is significant because even a single episode of hypoglycemia in the ICU may increase the odds of death in the hospital up to two-fold[6]. Thus the need to control glucose levels in the ICU must be balanced against the risk of hypoglycemia.

While the original study (showing the most positive outcomes) by Dr. Van den Berghe and colleagues used more accurate blood gas analyzers for all glucose measurements; the subsequent studies often used less accurate glucose meters for measurement of blood glucose. This has fueled considerable controversy over whether glucose meters, originally intended for use in diabetic patients to monitor glucose and dose subcutaneous insulin, are accurate enough to manage intravenous insulin in critically ill hospitalized patients[7, 8].

Traditionally, accuracy requirements for glucose meters were developed based upon the level of accuracy needed for safe and effective subcutaneous insulin dosing in the routine care of diabetes. These specifications are often visually displayed in an error grid, a tool developed by collecting the opinions of endocrinologists and other healthcare providers about the implications of various amounts of glucose measurement error on the safety and efficacy of subcutaneous insulin dosing. These error grid observations were codified in a set of guidelines issued by the International Organization for Standardization (ISO) and Clinical and Laboratory Standards Institute (CLSI) some years ago, and until recently used by some regulatory agencies as the measure of required glucose meter accuracy. One such commonly cited guideline, ISO 15197, required that 95% of glucose meter values fall within ± 15 mg/dL of the true or reference glucose value for serum glucose values < 75 mg/dL; and ± 20% of the reference value for serum glucose values ≥ 75 mg/dL[9].

Because glucose meter use in the hospital has changed as glycemic control strategies have changed, most experts now feel that the original ISO guideline is not appropriate as an accuracy guideline for hospital use glucose meters[7, 8]. To address these concerns, more stringent criteria for glucose meter accuracy have been proposed by both National Academy of Clinical Biochemistry (NACB) and CLSI. The guidelines are similar, and require 95% of glucose meter results to be within either ± 15 mg/dL (NACB) or ± 12 mg/dL (CLSI) of reference glucose for glucose values < 100 mg/dL, and within ± 15% (NACB) or 12.5% (CLSI) for glucose values ≥ 100 mg/dL)[10, 11].

The case against glucose meter use in the ICU

Several studies have documented that some glucose meters have limited accuracy when used on critically ill patients such as those on intravenous insulin in the ICU. The degree to which glucose meters correlate with laboratory glucose measurement varies between glucose meter technologies[12]; and correlation in the hypoglycemic and hyperglycemic ranges is poor for some meters currently available[13, 14]. In addition, patients in the ICU are on multiple medications, and often have abnormal hematocrit and/or oxygen tension, all of which may affect the performance of some glucose meters[12, 15, 16]. Finally, target glucose concentrations are narrower for this patient population than they are for patients using handheld meters to dose subcutaneous insulin, logically suggesting that improved accuracy of glucose measurement might be required. A number of studies have examined glucose meter accuracy and its impact on insulin dosing in the context of glycemic control, and concluded that glucose meters could not be safely and effectively used to manage critically ill patients on intravenous insulin in the ICU[13, 17, 18].

Because studies examining glucose meter accuracy in the ICU have been relatively small studies

using different meters and reference methods, the larger question of the impact of glucose meter error on patient outcomes during glycemic control remains difficult to address. The primary manner this has been overcome is by utilizing simulation studies to model the effects of various levels of glucose meter error on insulin dosing decisions and glycemic control.

Boyd and Bruns first established the use of simulation modeling as a tool to examine the relationship between glucose meter performance (bias and precision) and insulin dosing errors[19]. The initial study was based upon glucose values and insulin doses used for conventional subcutaneous insulin dosing for diabetic patients. The authors used Monte Carlo simulation to relate glucose meter bias and imprecision to insulin dosing errors during conventional subcutaneous insulin dosing. They found that glucose meters available at that time had sufficient accuracy and precision to avoid large insulin dosing errors in the context of traditional subcutaneous insulin dosing regimens[19].

Another study, designed to specifically model glucose meter use during glycemic control in the ICU, was based upon 29,920 observed glucose values among patients on intravenous insulin therapy in 2 ICU units within one healthcare institution. As expected, most of the values were in a narrow range of glucose value (102-135 mg/dL), such that insulin dose would change with every 20 mg/dL glucose increment according to the insulin dosing protocol in use. The authors found that allowing 20% total error in glucose meter measurements (previous ISO 15197 criteria) allowed for rare large (3 or more insulin dosing categories) insulin dosing errors; those that are most likely to produce hypoglycemia[20]. Decreasing allowable error to 15% eliminated large insulin dosing errors; but still allowed for 2-5% of insulin dosing decisions to be in error by 2 insulin dosing categories. Reducing error tolerance to 10% further reduced the rate of 2 category insulin dosing errors to less than 0.2%. The authors concluded that 20% glucose measurement error was not safe and effective for intravenous insulin dosing protocols that sought to maintain glucose values at normal or near-normal concentrations (tight glycemic control)[20].

After the publication of the NICE-SUGAR study, many institutions changed the glucose target values for ICU patients on intravenous insulin therapy to more moderate glucose values. To investigate whether glucose meter accuracy requirements for more moderate glycemic protocols differed from those suggested for tight glycemic control, the authors repeated the simulation studies using 25,948 observed glucose values in 1503 ICU patients on a moderate glycemic control protocol (110-150 mg/dL target value)[21]. Although the median glucose value was significantly higher among patients on moderate (134 mg/dL) compared to tight (116 mg/dL) glycemic control, most glucose values among patients on the moderate glycemic control protocol still fell into insulin dosing categories where insulin dose changed with every 20 mg/dL increment in glucose value. Rates of insulin dosing errors as a function of meter bias and precision were nearly identical to those predicted for the population of patients on tight glycemic control. This suggests that the observed relationship between glucose meter and insulin dosing errors can be generalize to insulin infusion protocols where insulin dose changes with every 20 mg/dL change in glucose value[21].

Simulation models suggest that 20% error is too much for glucose meters used to manage patients on intravenous insulin therapy. Because some studies of glucose meter accuracy in the ICU observed that glucose meter error exceeded 20% when used on critically ill patients[17, 21, 22], the simulation models have been used as evidence that glucose meters do not have the level of accuracy required for safe and effective

management of critically ill patients placed on intravenous insulin (glycemic control).

Only a small number of simulation studies have gone beyond relating glucose meter accuracy to insulin dosing errors; and attempted to relate meter error to the short-term patient outcomes such as rates of hypoglycemia, rates of hyperglycemia, or glycemic variability (rate and extent of change in glucose levels over time). One simulation model used a complex algorithm to predict the impact of glucose meter error over many days on rates of hypoglycemia, hyperglycemia and glycemic variability when glucose meter results were used to dose subcutaneous insulin in the context of diabetes self-management. The authors found that there was a threshold between 10-15% meter error that was predicted to result in increased incidences of hypoglycemia, hyperglycemia and increased glycemic variability[23]. One additional study used simulation modeling to assess the impact of both glucose measurement frequency and precision on predicted rates of hypoglycemia in the context of glycemic control in the hospital. Using hourly glucose monitoring to adjust insulin dose, the simulation model predicted that increasing imprecision above 10% CV would result in progressively increased rates of hypoglycemia (glucose < 60 mg/dL). The same simulation models suggested that using hourly glucose monitoring rates of hyperglycemia (> 160 mg/dL), time within intended target glucose range, and glycemic variability were all detrimentally affected when precision increased beyond 5-10% CV[24]. These studies differed in the type of insulin dosing modeled (subcutaneous vs. intravenous), glucose target ranges assumed, and frequency of glucose monitoring. However both raise concerns about the use of glucose meters to manage patients on intravenous insulin in the ICU. Both studies suggest a threshold effect of either glucose meter total error[23]or imprecision[24]; with a suggested minimum total error of 10-15% and imprecision of < 5%. Because a number of previous studies demonstrated total error greater than 10-15% when glucose meters are used on ICU patients[13, 17, 21], this has fueled concern about their use in this context.

The case for using glucose meters in the ICU

While studies of glucose meter use among critically ill patients have demonstrated both systematic differences (generally positive bias)[17, 25, 26] and variability[13, 14, 18] between glucose meter and laboratory glucose values, a few studies have concluded that the use of glucose meters during glycemic control may be appropriate. One study used Parke's error grid analysis to assess the clinical impact of glucose meter errors when arterial, venous or capillary samples were used to dose glucose meters. These authors concluded that glucose meters may be appropriate for use in glycemic control protocols when arterial or venous (but not capillary) samples are used[26]. However it is not clear whether use of the Parke's error grid is appropriate for assessing the clinical impact of glucose meter errors in the context of intravenous insulin therapy during ICU glycemic control protocols. Another study also examined differences between glucose meter and laboratory glucose when either arterial, venous or capillary samples from critically ill patients were used. This study examined the number and magnitude of insulin dosing errors when glucose meter (compared to laboratory glucose) results were used to make insulin dosing decisions using the institutional glycemic control protocol (target glucose 80-110 mg/dL). This study found that errors in the measurement of both venous catheter and capillary glucose resulted in more frequent large (2 or more insulin dosing categories) dosing errors; whereas use of arterial catheter whole blood on the glucose meter resulted in predominantly one category dosing errors[25]. Finally one study used consensus error grid and Bland Altman

analysis to study whole blood glucose accuracy using several different devices; and found that by limiting sample type to arterial blood that some glucose meters were accurate enough to be used during glycemic control[27].

In assessing the appropriateness of glucose meter use in the ICU, choice of sample type is an essential consideration. A number of studies have demonstrated that capillary glucose can be highly inaccurate in patients in shock, or patients with edema or poor tissue perfusion [13, 28-30]. Several studies have also demonstrated systematic overestimation of glucose values when venous catheters are used to obtain venous whole blood for analysis on some glucose meter technologies[31-34]. Arterial whole blood is very likely the best sample choice for monitoring whole blood glucose in critically ill patients. In considering the evidence for and against use of glucose meters in the ICU, one should pay special attention to sample source as a potential cause for poor glucose meter performance.

Other investigators have studied whether other factors may be more important than glucose monitor accuracy in determining the effectiveness of a glycemic control protocol. One study compared use of a standardized insulin infusion protocol to physician-directed intravenous insulin dosing in a mixed medical/surgical ICU. Use of the standardized infusion protocol reduced the rate of hypoglycemia from 16% to 4%, and also reduced the frequency of dextrose rescue[22]. Patients using the standardized protocol reached target glucose faster and maintained blood glucose in the target range (81-110 mg/dL) longer. Glucose in this study was monitored using capillary samples on a glucose meter, perhaps the least desirable sample for critically ill patients. Even with this limitation, the study demonstrated that execution of a standardized infusion protocol can improve at least short-term outcomes (hypoglycemia, time in therapeutic range)[22]. Another study demonstrated that by using an insulin infusion protocol that focused on velocity of glucose change (rather than absolute glucose levels), glucose meters could be used to maintain blood glucose in the range of 100-139 mg/dL with very little (0.3% of all glucose values < 60 mg/dL) hypoglycemia[35].

Another investigator has described a collaborative approach to establishing both glucose target ranges and insulin infusion algorithms based upon practice and nursing leader opinions about what could be safely accomplished. Using this approach they implemented an initial glycemic control protocol to keep glucose levels among critically ill patients below 140 mg/dL. They used hourly capillary glucose meter and/or laboratory serum/plasma glucose for all patients on intravenous insulin and observed a rate of severe hypoglycemia (glucose < 40 mg/dL) of 0.38%[36]. When staff in the ICU was comfortable with the “under 140” protocol, the target glucose range was decreased to 80-125 mg/dL with only a modest increase in severe hypoglycemia (0.92%). The authors concluded that by taking an incremental approach to glycemic control, starting with a higher target range and lowering the range only after staff demonstrated they could reliably execute the protocol, safe and effective glycemic control was possible using glucose meters for some monitoring[36].

A more common approach to improving outcomes during glycemic control is to use information technology solutions to computerize insulin doses based upon trended (rather than individual) glucose values. This approach mitigates the risk of hypoglycemia from a single aberrant glucose meter value. Using this approach one study demonstrated that rates of severe hypoglycemia were 4.25% when mostly capillary whole blood glucose meter values were used to dose insulin among 4588 critically ill patients managed on a glycemic control protocol with an 81-110 mg/dL target range[37]. These authors went on to investigate causes of hypoglycemia

among all incidents where glucose fell below 40 mg/dL. The authors found that ~ 70% of hypoglycemic episodes could be attributed to delay in obtaining glucose measurement; suggesting that human error (rather than measurement error) is responsible for the most insulin-induced hypoglycemia during traditional tight glycemic control protocols[37]. The same authors compared the computerized infusion protocol to a paper-based protocol and found that using a computerized protocol improved the time in therapeutic range, mean blood glucose level, and percent of blood glucose measurements below 70 mg/dL[38].

Finally a study over a one month period in three intensive care units at one institution found that using arterial whole blood to dose glucose meters, and relying upon consistent hourly glucose measurements performed by laboratory (rather than nursing) staff, rates of severe hypoglycemia were 1.4% despite a relatively low glucose target range of 80-130 mg/dL. In addition, 86% of severe hypoglycemic episodes observed were due to protocol violations (missed hourly glucose measurements or failure to change insulin infusion rate according to protocol instructions) [39]. When the glucose target range was changed to 110-150 mg/dL (with no change in glucose meter used or measurement frequency), no episodes of hypoglycemia were observed in 211 patients over one month[39]. A larger study (three months, 1503 patients) within the same ICU units found a rate of severe hypoglycemia of 0.25 % [21].

Collectively these studies highlight several key points that must be considered before determining the appropriateness of glucose meters for managing glycemic control in the ICU. The choice of sample type (arterial whole blood preferred) may be as or more important than the type of glucose monitor used for whole blood glucose measurement. Glucose meters have been used in effective glycemic control protocols demonstrating both low rates of severe hypoglycemia and reliable glycemic control in the ICU. Elements of effective protocols are computerized (rather than paper-based) insulin dosing algorithms, collaboration and teamwork to determine the appropriate glucose target for a given hospital or ICU population, and use of frequent (often hourly) arterial whole blood sampling for all patients on intravenous insulin.

The FDA draft guidance on glucose meter accuracy

While many studies demonstrating poor performance of glucose meters in critically ill patients used older glucose meter technologies, newer technologies with improved accuracy have recently become available[40-43]. Some recent studies have demonstrated that newer glucose meter technologies can meet even the more stringent CLSI POCT12-A3 accuracy guidelines (± 12.5% for values above 100 mg/dL) when used in the intensive care unit [41, 42]. Meters that meet more stringent accuracy guidelines such as POCT12-A3 would be performing within the 10-15% total error allowance predicted to minimize large insulin dosing errors in the context of ICU glycemic control. With the improved performance of newer glucose meters, one might think that the issue of glucose meter accuracy in the ICU was close to resolution.

To add fuel to the ongoing controversy about glucose meter use in the ICU, the Food and Drug Administration (FDA) released draft guidelines suggesting that improved accuracy was necessary for any future glucose monitors intended for hospital use. While the guidelines are still in draft form at the time of this review, FDA draft guidance criteria suggested that 99% of glucose meter values should be within 10% of the reference or true glucose value[44]. There is concern among some that tightening accuracy criteria to this level could impede the development of new meters and monitors, without improving

the quality of care delivered in the ICU during glycemic control.

Tips for point of care programs

Amidst this cloud of confusion and controversy surrounding glucose meter use in the ICU, what is the point of care program to do? First and foremost, consider the entire glycemic control protocol in use within your institution, and the role that glucose meters play in the overall scheme of glycemic control. Eliminating the use of glucose meters in support of intravenous insulin protocols, without first considering alternatives and implications, would almost certainly have an adverse effect on patient care. Understand the effectiveness of the glycemic control protocol (rates of hypo and hyperglycemia, time within intended glucose range) as implemented, and the systematic issues that may be leading to adverse outcomes such as hypoglycemia. If the major issues are remembering to obtain glucose values in a timely manner to facilitate insulin dosing decisions, or communicating glucose results to providers in a timely manner, then changing glucose measurement devices (especially away from the bedside) would not be expected to improve outcome. If spurious glucose results have been observed in some ICU patients, determine whether common interferences (low hematocrit, some medications) in the ICU environment may be affecting the glucose meter technology in use. If user errors such as incorrect strip codes or under-dosing of strips are suspected; consider switching to a glucose meter technology that reduces the likelihood of these errors and examining training and competency systems.

Hospitals and point of care programs should also consider the sample type (capillary, arterial or venous whole blood) routinely used for bedside glucose measurements, before making a decision to switch technologies or glucose measurement devices. If capillary sampling is being used as the predominant sample type, switching to arterial whole blood may improve measurement accuracy without requiring large changes in workflow or testing processes. Finally, consider evaluating the accuracy of the device being used by comparing whole blood glucose meter values to laboratory serum or plasma glucose obtained from ICU patients. If the vast majority of glucose meter values are not within 15% of lab glucose values, then it is likely that more accurate glucose measurements are both possible and desirable.

CONCLUSION

Glucose meter use in the ICU environment will continue to be a controversial issue. Simulation models have provided the best evidence available to relate glucose meter accuracy to insulin dosing errors during glycemic control in the ICU. However they do not provide a way to measure the impact of glucose meter error on patient outcome. Studies directly relating glucose monitor accuracy to glycemic control outcome (mortality, infections, transfusions, etc) or effectiveness (hypoglycemia, hyperglycemia, time in therapeutic range) are needed to understand the level of glucose meter accuracy required for management of critically ill patients on intravenous insulin therapy.

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Infection transmission associated with point of care testing and the laboratory's role in risk reduction

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ABSTRACT

Lack of knowledge and confusion exists regarding safe and appropriate use of blood glucose monitoring equipment. Increasing numbers of diabetics, and exponential growth in blood glucose monitoring presents increased opportunities for infection transmission between patients. Diabetics have increased exposure to blood and blood borne pathogens from frequent blood glucose monitoring.

Risk factors have been identified in infectious outbreaks and by analysis of testing practice. Point of care blood glucose meters are frequently contaminated by blood. Bacterial and viral organisms survive on surfaces and in dried blood. Instrumentation is shared between patients, and is heavily utilized in institutional settings, so that serial testing is performed on multiple patients within a short timeframe. Hand hygiene, glove changes and meter disinfection between testing events has been found to be inconsistent. Time pressure for meter usage competes with proper cleaning and disinfection procedures. Meter storage areas are frequently contaminated by blood. Multi-use lancets, improperly used for serial patient blood sampling, are a source for infection transmission. Test strips in vials, frequently contaminated by bacterial organisms, present potential hazard. The responsibility of the clinical laboratory is to insure successful implementation of practices that insure patient safety.

Risk reduction strategies include single-use auto-disabling skin

puncture devices for blood sampling; hand hygiene and glove change for every testing event; effective meter cleaning and disinfection for every testing event; meter use restriction to a single patient; safe practices for glucose meter storage; infection control practices to reduce contamination of blood glucose test strips or changes in test strip packaging and test strip dispensing.

POINT OF CARE GLUCOSE MONITORING IS ON THE RISE

Increasing numbers of newly diagnosed diabetics and increasing overall prevalence of diabetes in the U.S. population herald an increasing number of individuals for who point of care (POC) blood glucose monitoring is performed. Current United States Centers for Disease Control and Prevention (U.S.CDC) estimates are that 25.8 million people in the United States, or 8.3 % of the population, have undiagnosed or diagnosed diabetes[1]. Whether the diabetic patient is prescribed nutritional modification, oral medications or insulin therapies, blood glucose monitoring (BGM) continues to be the foundation of diabetes management. The vast majority of diabetics -for example, approximately 86% of diabetics in the U.S. – are monitored monthly or more often[2]. Point of care glucose testing is therefore one of the most common tests performed in hospital, ambulatory and home settings.

If diabetics perform self-monitoring why are they at increased risk for hepatitis B?

Patient-to-patient transmission of infections such as hepatitis B can be transmitted through point of care devices, such as blood glucose meters. In self-monitoring of blood glucose (SBGM), an individual performs the entire testing process for themselves[3]. Two-thirds of diabetics perform SBGM[4] . The great majority of health care institution-associated hepatitis b outbreaks have been associated with assisted blood glucose monitoring (ABGM)[5]. In ABGM, the steps of blood glucose testing are performed by a caregiver for an individual or a group of individuals[3]. ABGM occurs in a variety of patient care settings: acute care hospitals, clinics, skilled nursing facilities, long term care and residential care settings. ABGM is also provided to self-monitoring diabetics at school or camp, during acute hospitalizations, in rehabilitation facilities, and at ambulatory care visits The risk for infection transmission exists wherever blood glucose monitoring equipment is shared, and/or where those performing tests do not follow consistently follow basic infection control practices: long-term care facilities; acute care facilities; clinics; health fairs; shelters; prisons; senior centers; and schools and camps.

Bacterial and viral pathogens can be transmitted from equipment to patients. The primary focus on infection transmission linked to point-of-care testing is viral disease, most notably hepatitis B (HBV), hepatitis C (HCV), and human immunodeficiency virus (HIV), though bacterial transmission is also of concern. The emphasis on hepatitis B risk in particular is based on epidemiology of outbreaks[6], as well as a higher infectivity rate (approximately 30% attack rate following exposure, versus 0.2% for HIV, and 3% for HC)[7].

Quantifying the risk of hepatitis B in diabetics

Adult diabetic individuals are at significantly higher risk for hepatitis B infections than non-diabetic individuals. The increase in risk of hepatitis B infection for diabetics is associated with blood exposure. An investigation of the relative risk of acquiring hepatitis B in 865 adult diabetics who did not harbor other risk factors for hepatitis B demonstrated the odds of contracting acute hepatitis B were 2.0 times higher for diabetics less than 60 years of age; and 1.5 times higher for diabetics greater than or equal to 60 years

of age[8]. Seroprevalence studies demonstrated a 60% increase in antibody to hepatitis B core antigen, or anti-HBc, among non-institutionalized adults with diabetes, compared with non-diabetics ($p<0.001$).The risk differed by age group: at 18-59 years of age, diabetics showed a 70% increase ($p< 0.001$) of hepatitis B exposure compared to non-diabetics, whereas diabetics greater than or equal to 60 years of age showed a 30% increase ($p= 0.032$)[8]. In the United States, this increased risk has prompted public health agency epidemiologic investigations, outreach efforts promoting best practices by public health agencies and public health initiatives such as a hepatitis B vaccination campaign for diabetics[8].

Analysis of U.S. outbreaks of hepatitis B associated with blood glucose monitoring reveal that outbreaks have occurred with increasing frequency over the twenty years audited (1990 through 2009) [9]. Outbreaks have resulted in patient deaths[10]. The unsafe practices most frequently implicated in these outbreaks at this time are spring-loaded finger stick lancet devices used on multiple individuals, and omission of cleaning and disinfection of blood glucose meters between patient testing events[6]. Other supplies and components of the testing process have not been noted or as well studied.

Proper choice and use of single-use, auto-disabling skin puncture or lancet devices for blood sampling

One of the most serious biohazard risks to patients undergoing point of care testing is the use of finger stick devices on multiple patients. Molecular genotyping has even provided evidence of disease transmission in a hepatitis B outbreak by a lancet cap[11]. Because of this risk, the CDC and United States Food and Drug Administration (FDA) recommend that finger stick devices should never be used for more than one patient[12.] It is further recommended that patients and health care professionals adopt the immediate precaution of using auto-disabling, single-use finger stick devices for assisted monitoring of blood glucose. These devices are designed to be used only once, after which the blade is retracted, capped or otherwise made unusable. These are sometimes called "safety" lancets[13]. Design of safe practices for residential and other similar settings where a patient will be using their own reusable finger stick device is also critically important, such as proper labeling with the patients name and securing the lancet in a safe place (such as in their room) to protect from inadvertent use by or for others.

Hand hygiene and glove change requirement for every testing event

Best practice, according to public health agencies, is a mandatory change of gloves and hand washing after each and every testing event[14]. Even in the absence of visible blood, infectious pathogens can be transmitted through indirect contact transmission. Gloves, like hands, carry flora or blood from surfaces and from patients touched. As is required for venipunctures, when performing finger sticks, gloves should be changed between patients[3]. If hand hygiene and glove changes are not consistently performed between patients, device contamination and disease transmission (e.g., hepatitis B) can occur. The FDA advises "Change gloves between patients, even when using patient-dedicated POC blood testing devices and single-use, auto-disabling finger stick devices."[13]

Effective meter cleaning and disinfection requirement for every testing event

Best practice is to clean and disinfect the meter after each and every use, for meters designated for multi-patient use. A high rate of blood contamination of glucose meters raises the risk of blood-borne pathogen transmission. A multicenter study assessed meter contamination in institutions by evaluating 609 meters across a variety of care units. Presence of blood was evaluated first by visual inspection; followed by

a reduced phenolphthalein test for hgb. Overall, mean meter contamination rate was 30.2% (±17.5%)[14]. Of 12 hospitals surveyed, only one routinely cleaned meters between patients. Sharing of blood glucose meters should be avoided, if possible. If shared, the device must be cleaned and disinfected after every use according to manufacturer's instructions. If there are no manufacturer's instructions, the device must not be shared[12].

Selection of appropriate products and use of recommended procedures for cleaning and disinfection of point of care devices is critical to reduce risk of infectious cross-contamination. The use of 70% alcohol wipes is inadequate for disinfection. According to the FDA: "The disinfection solvent you choose should be effective against HIV, Hepatitis C, and Hepatitis B viruses ... Please note that 70% ethanol solutions are not effective against viral blood borne pathogens and the use of 10% bleach solutions may lead to physical degradation of your device."[15]

Identify patterns of use of point of care devices which pose hazard to patients

Risk factors for patient safety have been identified by analysis of testing practice. Instrumentation is shared between patients, and is heavily utilized in institutional settings, so that serial testing is performed on multiple patients within a short timeframe. A study of blood glucose meter use in a 214-bed acute care hospital demonstrated that, over a 31-day baseline period, 11,665 glucose measurements were performed on 803 patients using 38 glucose meters. Sequential tests were performed on different patients using the same meter within 24 hours in 9302 of 11,665 (79.7%) tests: 99.9% were performed within 24 hours and 60.9% were within 1 hour[16]. Time pressure for meter usage competes with proper cleaning and disinfection procedures. Inadequate time for thorough cleaning and disinfection between patients poses a safety risk. Clearly, if multiple point-of-care devices are used on a single patient, and without a use restriction, all patients on a unit could be tested with all the meters over a short time interval. As previously cited, independent published literature indicates inconsistent and/or ineffective meter cleaning practices. Without appropriate and consistent meter cleaning and disinfection, increases risk for blood borne pathogen exposures.

Dedicated meter assignment to an individual patient

To reduce the risks associated with point of care testing, The CDC and FDA recommend that each glucose meter should be assigned to a single patient whenever possible. This guidance extends also to other point of care devices also[12]. If dedicating POC blood testing devices to a single patient is not possible, the devices should be properly cleaned and disinfected after every use as described in the manufacturers' product device labeling and instructions[12].

Safe practices for glucose meter labeling and storage

If meters are not effectively cleaned and disinfected after every use, storage may present additional risk of cross-contamination by blood. In a survey of blood contamination of glucose meters, a mean of 20% of hospital meter storage areas were contaminated. Up to 52.7% of storage areas in institutions were contaminated by blood[14]. Analysis of meter labeling storage procedures is good practice to protect patients from cross-contamination. If a dedicated meter for single-patient use is provided, such measures can help protect patients from inadvertent use of their meter by others[12].

Recent evidence indicates bacterial contamination of blood glucose test strips requires intervention

In a study conducted over six weeks in four United Kingdom hospital wards, the bacterial load on 148 glucose test strips was quantified by culture. The overall test strip contamination rate ranged from 16.6% - 35.7%. Enteric and skin flora were the bacterial species identified. The authors noted that the narrow test strip vial opening requires repeated manual touching to pull a strip out, under non-sterile conditions. Investigators' recommendation was to "dispense single units that can be used in a 'no-touch' procedure"[17] A second, multicenter evaluation of glucose test strip contamination found that the majority of open vials in use in five hospitals had contaminated glucose test strips. In this U.S.-based study, between 27-70% of opened vials tested positive for bacteria, regardless of vendor, versus only 0-4% of individually foil-wrapped strips. Test strips were culture-positive for a variety of bacterial (enteric and skin flora) species[18]. A third study , based in three hospitals in Spain, tested 423 test strips which had an overall contamination rate of 34% (146/423). Comparing contamination rate and differences in test strip packaging, the authors found that 7% of individually-wrapped strips were contaminated, versus 45% of strips from multi-use vials ($p < 0.001$). Pathogenic organisms such as methicillin-resistant *Staphylococcus epidemidis* and *Staphylococcus hemolyticus* were recovered from multi-use vials but not from the individually-wrapped strips[19]. The latter two studies were industry-sponsored studies. Confirmation by independent investigators would be a valuable addition to this growing literature .

Relevant CDC guidance is the following general recommendation : "Unused supplies and medications taken to a patient's bedside during finger stick monitoring or insulin administration should not be used for another patient because of possible inadvertent contamination".[20]

The proposal to address test strip contamination by dedicating individual vials to single patients clearly adds cost, due to the mandatory discard of unused test strips upon patient discharge. In addition to increasing health care cost, assignment of a test strip vial to an individual patient may not eliminate contamination risk. Noteworthy is the U.K. finding, where (independent) investigators found that opened vials that stayed with a single patient had same contamination rate as those that moved from room to room[17]. What are the financial consequences of discarding unused strips from common-use testing vials? A real-life estimate of the financial impact of strip vial wastage was undertaken to answer this question. Based on a set of assumptions of patient census, glucose test workload and hospital length of stay, such estimates may be calculated for a given institution. In this independent published study, the author estimated the annual cost of test strip wastage to range from $80,000 USD with 25-strip vials to more than $170,000 USD with 50-strip vials. This study highlights that – if single-use test strip vial is adopted - choosing glucose vendors and/or test vial count (e.g., 25 versus 50 count test strip vials, or single-use packaging versus multi-strip vials) has potentially substantial, largely unrecognized, financial impact[21]. Individually foil wrapped test strips additionally protect against moisture and environmental contamination, considerations outside the scope of this paper. However, not all vendors have offered this product as yet, and a solution to this problem must be found across multiple vendor products.

Bacterial test strip contamination may be addressed risk by sterile handling protocols, albeit with addition of time and inconvenience to the overall testing process. Alternatively, test strip contamination could possibly be reduced in the future by single-unit, "on demand" test strip

dispensers (e.g. a "touch less" technology) and/or industry-wide transition to single test strip packaging. The principle of single-unit dispensing and/or packaging has become the norm of pharmaceuticals, health care supplies and other patient equipment.

SUMMARY

The number and scope of infectious outbreaks associated with blood glucose testing points to knowledge gaps and confusion regarding the appropriate, safe use of blood glucose monitoring equipment. Educational campaigns by public health agencies (e.g. CDC and FDA) and professional societies such as the IFCC and College of American Pathologists serve to inform responsible parties in health care settings. Device manufacturers are responsible for improved, effective, validated cleaning and disinfection protocols, product labeling, and package instructions. The following strategies can help reduce the risk of infection transmission between patients during point of care testing: using only single-use auto-disabling skin puncture/lancet devices for blood sampling; requiring hand hygiene and change of gloves between patients for each testing event ; effective meter cleaning and disinfection for every testing event; advocating for restriction of meter use to a single patient, when possible; properly labeling and storing meters, such that risk of inadvertent use for/by other patients is eliminated; and reducing contamination rate of glucose test strips in vials by employing sterile practices entering and removing test strips from vials, or by making changes in test strip packaging and dispensing., It is our responsibility to use these best practices to help protect patient safety.

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Primary hypoparathyroidism misdiagnosed as epilepsy - a case report

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Running title*:*
Seizures, hypocalcemia and cerebral calcification.

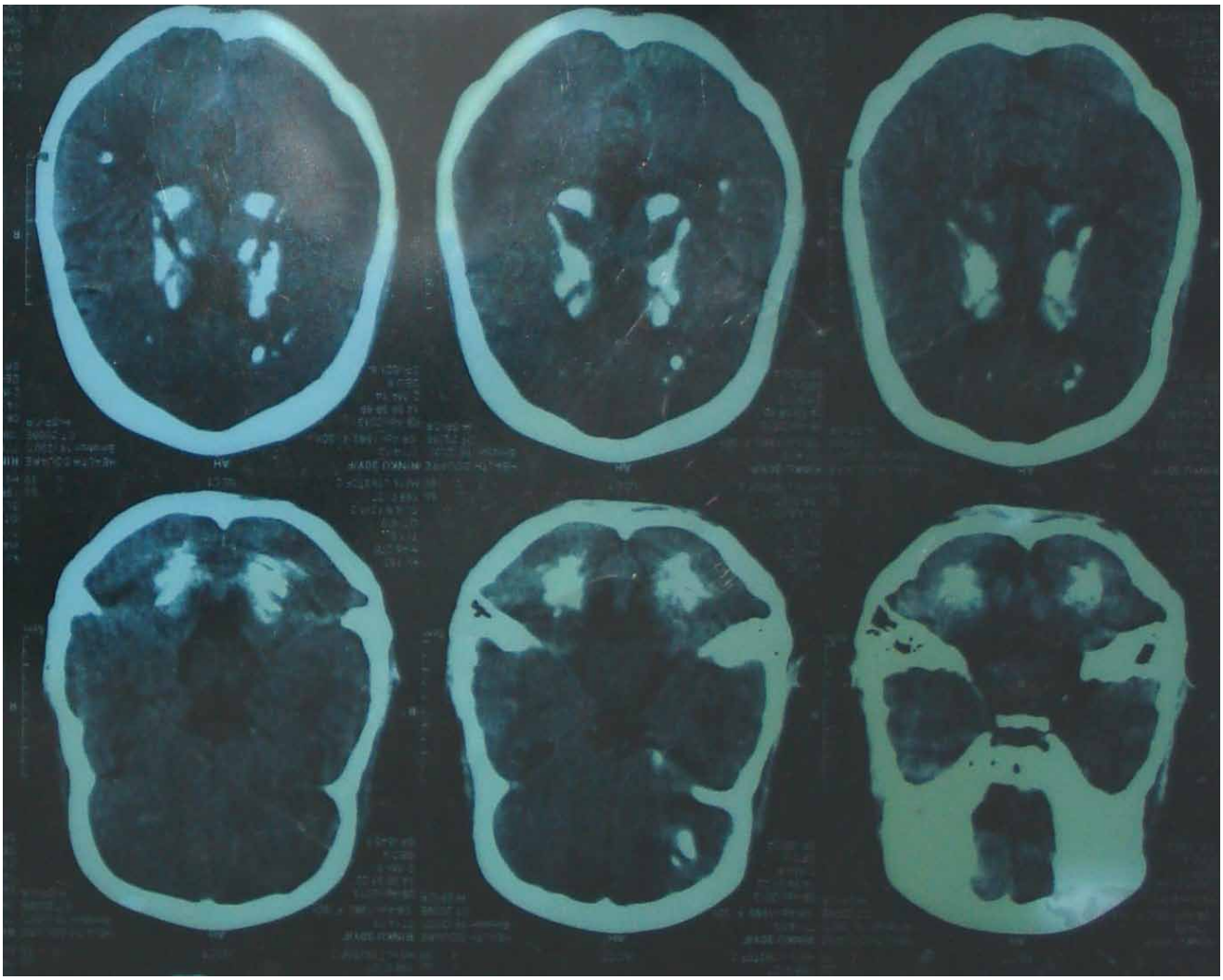
ABSTRACT

Absent or inappropriately low intact parathyroid hormone along with hypocalcemia is the diagnostic criterion of hypoparathyroidism. Clinically, hypoparathyroidism manifests predominantly as neuromuscular dysfunction caused by hypocalcemia. We present here a case of hypoparathyroidism wrongly and ineffectively treated as epilepsy for four years prior to reporting to our hospital. Hypoparathyroidism was diagnosed in our patient on the basis of low serum calcium (ionized and total), high phosphate and very low IPTH levels in face of normal magnesium levels along with radiological evidence of cerebral calcification. The authors stress on the need to include hypoparathyroidism in the differential diagnosis of seizures and the need to treat with 1, 25 dihydroxycholecalciferol.

CASE REPORT

A 30 year old female presented to the neurology out patients department for cramps, rigidity, tremor and twitching and abnormal movements of hands and feet. The patient complained that this problem was continuing despite treatment for 6 years. On checking her old prescriptions and reports, it was found that she was being treated with antiepileptics [eptoin 100mg bid] along with vitamin E [Evion400 mg od] .A CT scan of brain (done outside) revealed basal ganglia calcification [Figure 1]. She was referred to Endocrinology department .While in the OPD; two bouts of tetany were witnessed by the doctor, followed by recovery in a few minutes. She was married for twelve years, had her first child six years back, who died at 6 months of age due to high grade fever. Two years later, she had a son. Six months after delivery, she began to experience convulsions, tetany, stiffness of hands and feet and would fall down, and then recover completely on her own, in a few minutes. This happened about twice or thrice a month. She had long standing generalized weakness. She was employed in a bangle making factory, and had to give up her job due to this recurring health problem.

Figure 1 Non contrast CT scan of brain showing calcification



On examination, she weighed 32 kg, height 144 cm, and blood pressure 90/60 mm Hg. All other systems were normal on examination. Findings from an electroencephalogram were normal. Her laboratory investigations were as follows [reference range in parantheses].Serum calcium was 5.39 mg/dl [8.4-10.2], ionized calcium at 0.7 mmol/L[1.12-1.32], 24 hour urine calcium 259 mg [100-300], thyroid stimulating hormone [TSH] 6.73 µ IU/ ml [0.25-5], intact parathyroid hormone [IPTH] 13.54 pg/ml [15-65],phosphorus 7.57 mg/dl [2.5-4.5], magnesium 1.9 mg/dl [1.6-2.5], hemoglobin 10.5g/dl [12-15], fasting plasma glucose 100.4 mg/dl [70-110]. Albumin and 25 hydroxyvitamin D levels were within reference range. No nutritional, familial, congenital, infiltrative or autoimmune cause of hypoparathyroidism was obvious. Our tests for ANA and APLA by IFA and ELISA respectively tested negative Patient never had surgery or irradiation of neck. Eye examination revealed no abnormality. Cortisol level was within reference range, excluding hypoadrenalism. On clinical examination, there was no evidence of mucormycosis or any other fungal infection

The patient was diagnosed as primary hypoparathyroidism and treated with activated vitamin D [1, 25 dihydroxycholecalciferol].Three months later, her calcium level is 8.9 mg/dl and phosphate 5.2 mg/dl, intact parathyroid hormone is 15.9 pg/ml [15-65] and she has not experienced the seizures since two weeks. She has also regained her happiness and confidence to get back to her livelihood again.

DISCUSSION

Hypocalcaemia may be an asymptomatic laboratory finding or a life-threatening metabolic disturbance. The clinical presentation of hypocalcaemia in hypoparathyroidism is usually insidious and classical symptoms may be absent, even in patients with profound hypocalcaemia.[1] Its prevalence is 18% in all patients in hospital and 85% in the intensive care unit [2] The clinical algorithm for the workup of the patient who presents with hypocalcemia [3] aims to differentiate hypocalcemia associated with an absent or inappropriately low serum parathyroid hormone concentration (hypoparathyroidism) from hypocalcemia associated with an appropriate compensatory increase in parathyroid hormone. Transient hypoparathyroidism with biochemical abnormalities is commonly seen (>83% of cases) after thyroid surgery. [4] However, our patient had no recent or remote history of thyroid/neck surgery or irradiation. Magnesium level of our patient was normal, which ruled out nutritional deficiency.

Basal ganglia calcification occurring in idiopathic hypoparathyroidism, correlates with the duration of hypocalcaemia, choroid plexus calcification, seizures and cataract and has been observed to worsen despite maintenance of normal calcium levels. [5] The culprit is believed to be the high serum calcium-phosphorus product ratio and poor calcium control. A literature review of the clinical presentations of basal ganglia calcification revealed that there are diverse presentations, the most common including seizures, mental deterioration, and disorders of cerebellar or extra-pyramidal function. Movement disorders, chorea, or parkinsonism are present in 20 - 30% of patients with basal ganglia calcification, while some patients are asymptomatic [6] Decreased PTH level and hypocalcemia exclude other causes of intracerebral calcifications like pseudohypoparathyroidism, hyperparathyroidism, monoxide carbon intoxication, encephalitis, Fahr disease, idiopathic basal ganglia calcifications, Cocayne syndrome, tuberous sclerosis, neurofibromatosis, vascular disease (vascular malformations, chronic ischemic or hemorrhagic stroke), cerebral parasitosis [7] Our patient's recovery from tetany with vitamin D and calcium, absence of family history of similar features and biochemical test

results helped rule out Fahr's syndrome [8] Due to financial constraints, no genetic testing could be done.

In a prospective study, Aggarwal and colleagues found there was a significant association between cognitive dysfunction and the duration of hypocalcemia, serum calcium levels, and calcium-phosphorus complex formation, but no association with serum 25(OH) D levels, serum PTH levels, or the volume or site of basal ganglia calcification. [9]

Presently, treatment consists of calcium supplementation and the use of vitamin D analogs, but PTH replacement is under investigation. [10] Oral calcium and vitamin D restore the overall calcium-phosphate balance. [11]

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Polish Code of Ethics of a Medical Laboratory Specialist

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ABSTRACT

Along with the development of medicine, increasingly significant role has been played by the laboratory diagnostics. For over ten years the profession of the medical laboratory specialist has been regarded in Poland as the autonomous medical profession and has enjoyed a status of one of public trust.

The process of education of medical laboratory specialists consists of a five-year degree in laboratory medicine, offered at Medical Universities, and of a five-year Vocational Specialization in one of the fields of laboratory medicine such as clinical biochemistry, medical microbiology, medical laboratory toxicology, medical laboratory cytomorphology and medical laboratory transfusiology.

An important component of medical laboratory specialists' identity is awareness of inherited ethos obtained from bygone generations of workers in this particular profession and the need to continue its further development. An expression of this awareness is among others Polish Code of Ethics of a Medical Laboratory Specialist (CEMLS) containing a set of values and a moral standpoint characteristic of this type of professional environment. Presenting the ethos of the medical laboratory specialist is a purpose of this article. Authors focus on the role CEMLS plays in areas of professional ethics and law. Next, they reconstruct the Polish model of ethos of medical diagnostic laboratory personnel. An overall picture consists of a presentation of the general moral principles concerning execution of this profession and rules of conduct in

relations with the patient, own professional environment and the rest of the society. Polish model of ethical conduct, which is rooted in Hippocratic medical tradition, harmonizes with the ethos of medical laboratory specialists of other European countries and the world.

ETHOS OF THE MEDICAL LABORATORY SPECIALIST

Polish Code of Ethics of the Medical Laboratory Specialist

The turning point of the 20th and 21st century, which was characterized by particularly rapid development of medicine, genetics and biology, became a time when the autonomy of the medical laboratory specialist profession was formed [1]. In Poland, the *Act from 27 July 2001 about the clinical diagnostics* [2] legally sanctioned the medical laboratory specialist as the fourth important profession in the group of medical professions such as the doctor, the pharmacist and the nurse. The growth in importance of the clinical diagnostics, which enables taking effective therapeutic actions, monitoring illnesses and conducting medical prevention, resulted in granting the medical laboratory specialist the status of the profession of the public trust [3]. The public trust enjoyed by representatives of this profession imposes a special obligation to be guided by high moral standards. The problem of ethics of the medical personnel of diagnostic laboratories was repeatedly discussed in the literature devoted to the subject [4-9].

The purpose of this article is to present the ethos of the medical laboratory specialist in the Polish Code of Ethics of the Medical Laboratory Specialist (CEMLS) [10]. Under the notion of "ethos" authors understand particular moral attitudes characteristic of a specific social group which result from the affirmation of certain values. The Code of Ethics is an expression of certain maturity of the "system of customs" and moral awareness of Polish medical laboratory specialists. This pioneering ,on a world scale, document was approved on 13 January 2006 during the Extraordinary Domestic Meeting of Medical Laboratory Specialists. In order to popularize it also amongst medical diagnostic laboratories staff of other countries, it was translated into English [11] and French [12]. The presentation of the Polish model of conduct of the medical laboratory specialist found in CEMLS is preceded by some observations on the role of this Code in the area of professional ethics and its place in the legal system. Characteristics of the ethos of the discussed profession were presented in four dimensions. First, there is the analysis of general rules of work in the medical diagnostic laboratory. Further aspects show the medical laboratory specialist in relation to the patient, their environment and the rest of society.

Role of CEMLS in professional ethics

CEMLS is part of a dispute, which has been going on for years, concerning the role of codes in professional ethics. Two extreme views clash – those represented by supporters of "code ethics" and those represented by the adherents of "no-code ethics" [13]. Opponents of the codification of ethics [14] usually formulate three accusation claims: deontologism, conventionalism and opportunism. The first one is based on the statement that world of the values and duties won't ever be transformed into neat manual of the moral conduct. The code of ethics reduces the problem of the responsibility to the obedience to norms. Evaluation criterion is established as doing one's duties, rather than personal reflection or examination of one's conscience. The second claim comes out from the statement that the morality is something independent of the convention and contract, and professional ethics is inseparably connected with it. Creating a code causes the problem of

establishing standards in professional ethics: who and by what criteria is supposed to appoint these norms? A sign of opportunistic character ascribed to supporters of codes is expediential dimension of those documents. Elaborating codes usually serves a specific occupational group rather than develops a broad and impartial moral reflection.

In a response to accusations of supporters of "no-code ethics" opposite arguments are put forward. Firstly, they underline that obedience to the code is never discharging an individual from moral responsibility. Secondly, the norm included in the code, irrespective of the convention in which it was created, is additionally sensitizing the employee to the moral dimension of action to which this norm refers to. Thirdly, codes of ethics very often appeal to anti-pragmatic category of dignity. It is hard to accuse these documents of exclusively economic character and to assign to them only praxeological function. It is possible also to dismiss the accusation of the opportunism by filling the elementary requirement put before every code of ethics, i.e. protecting the social welfare. True concern about the society as a whole protects from the situation in which the business of a given occupational group will become the only grounds for creating the code [15].

In the light of this discussion it is possible to express two significant conclusions. It is hard to imagine professional ethics without clearly defined principles and duties and those are most often expressed in the form of norms of the code. This does not mean though that the entire area of professional ethics is reduced and is contained in these documents. Art. 27 of CEMLS accurately emphasizes it: "this Code of Ethics of a Medical Laboratory Specialist is *the collection of fundamental ethical standards* that should be followed by each representative of the profession" [11]. Secondly, codes should not become a "legalization of ethics". Contrarily, the point is that norms included in codes are rooted in the value systems of the community. This rooting of CEMLS is explained in the preamble: „The Code of Ethics of the Medical Laboratory Specialist is grounded in generally accepted ethical standards as well as the principles originating from the professional tradition" [11]. Concern about "legalization of ethics" in CEMLS is dispelled by Art. 28-29: „This Code of Ethics of a Medical Laboratory Specialist is the source of moral guidelines and does not replace the process of a medical laboratory specialist's personal and professional development. Continuous reflection on the principles of conduct of the medical laboratory specialist should constitute the grounds for the improvement of moral and professional attitudes of medical laboratory specialists" [11]. In this context it is possible to agree with Skuczyński who writes: "not the very existence of codes of ethics is dangerous, but reducing ethics to code decisions. Neither the deontology, nor the conduct of individuals can be rational or irrational exclusively on account of codes of ethics, though norms contained in them can constitute arguments in practical reasoning - never though the only ones" [16]. Recognizing the need of creating codes of ethics and legitimacy of the CEMLS study, it is worthwhile to pay attention to the issue of their more or less legal character, i.e. their relation to the constitutional law.

Legal character of CEMLS

The evaluation of legal character of the code of ethics in a given country is significantly influenced by its legal tradition. Generally as part of the Anglo-Saxon tradition it is possible to assign far more features of "ordinary" law than in the tradition of the European continent [17]. In the United States codes of ethics have a character of the law or similar to the law, above all on account of the possibility of enforcing them. Norms included in these codes are not only a

basis of disciplinary liability, but also of other kinds of legal liability. European codes of ethics usually contain general norms on execution of a given profession and are less legalistic and less formal than their American equivalents. One should however remember that also in part of the Old Continent their norms belong to legal systems and as such constitute the basis of disciplinary or professional liability.

CEMLS has it legal authorization in Art. 44 of the *Act of the clinical diagnostics* [2], which imposes "codifying principles of ethics of medical laboratory specialists". This fact does not dispel all doubts concerning legal character of the document in question. In the discussion present for many years in Poland (similarly as in other countries) about the legal status of codes of ethics it is possible to exemplify two outermost positions. First are supporters of the monism, regarding the law as the only normative category. They claim that a code of professional ethics based on provisions of a relevant act becomes a part of the legal system. In contrast, dualism maintains the existence of a second normative system besides the law, which is described as the sphere of moral, ethical or deontological norms. In this understanding the issued code pursuant to the provisions of the above mentioned act retains its identity and is not an object of incorporation in the legal system. The ethical norms included in the code do not have a legal status, but re-describe norms of the constitutional law. The statements of the Polish Constitutional Tribunal [18,19], concerning the Code of Medical Ethics can prove that in Poland this dualistic model is the model in force. However, there are increasingly frequent attempts to reconcile both positions by treating norms of professional ethics as specific norms of "soft law" as opposed to traditional "hard law".

The attempt to rank codes of professional ethics as "soft law" is justified by the exceptional character of these documents. Their appropriate objective is the regulation of moral duties of representatives of a given profession. It seems that they are in the middle "between the law and the conscience" and thus they have certain features of both law and morality. Codes constitute an expression of community of values of a given occupational group and are an effect of the process of self-regulation. With regard to the law they have subjective character and with regard to conscience (of individual representative of a given profession) an objective one. It is also possible to say they have a double nature. On one side, documents of this type "soften" legal norms, on the other, "harden" standards of proceedings rooted in individual's sense of morality. Skuczyński postulates we should "rank codes of ethics to soft law understood as a particular type of the social control" [16]. The typical features of this type of control are: the specific way of constituting it in the form of self-regulation, the character of applying it in non-formalized procedures and the connection above all with "soft" sanctions, e.g. punishments of a disciplinary character. It seems that one should treat Polish CEMLS as a document of „soft law" as it has all three above mentioned features. Firstly, it was developed by National Chamber of Medical Laboratory Specialists (NCMLS) as a form of self-regulation of this environment. Secondly, formalized procedures of its implementation do not exist as opposed to only a general statement saying that „the responsibility for the execution of the principles and provisions of the Code of Ethics of a Medical Laboratory Specialist is vested in the authorities of the Corporation and, in particular, the members of its Ethics Committee" [11]. Finally, it constitutes an important point of reference in the ruling of the Disciplinary Body.

General principles of professional practice

An attentive reading of CEMLS allows to discover the Polish model of the medical laboratory

specialist's ethos. Art. 1 of the Code lists three areas every representative of the discussed profession must take into account in their everyday work: „Medical Laboratory Specialist shall carry out his/her tasks in a manner compliant with generally accepted ethical standards, the principles of professional practice as well as the provisions of law which regulates professional performance" [11]. The first of the aforementioned matters is the space of ethics and, contained within, widely accepted norms of conduct. The preamble and subsequent articles of the Code shed some light on the notional scope of the term "generally accepted ethical standards". Authors of the Code regard the good of the human person, which should be protected in both the individual and the social dimension, as the principal ethical norm. The uniqueness of every human person justifies the fundamental role of that good. Respect for the human dignity finds its due expression in an honest and solid service to the patient. That task can be enabled by taking into account in the everyday work values such as: the good, the truth, the freedom, the equality and justice.

The medical laboratory specialist should carry his work out according to "the principles of professional practice". Taking into consideration the fact that medical diagnostics used to be performed by doctors, the rules of conduct of the medical laboratory specialist are rooted firmly in the Hippocratic tradition [20]. In the canon of "principles of professional practice" one can boldly rank, among others, performing all activities with respect for the life and health of the patient, keeping professional secrecy, conscientiousness, reliability and honesty. At present, increasingly comes to the fore also the principle of labor economics. All examinations conducted by the medical laboratory specialist should fulfill the highest standards which rely on the up-to-date knowledge. In most European countries, including Poland, the desired quality of laboratory test results is ensured by application of standards of *International Organization for Standardization* (ISO). By 2004, Polish medical diagnostic laboratories introduced a general standard of PN-EN 17025:2001 concerning competence of research and calibration laboratories and from 2005 European norm dedicated for medical laboratories: EN 15189:2003 [21,22].

The third area according to which every medical laboratory specialist should act is the set of „the provisions of law which regulates professional performance". In Poland the basic document in this regard is aforementioned *Act on the clinical diagnostics* [2]. It regulates the terms and conditions of the medical laboratory specialist profession as well as the issue of disciplinary liability for malpractice. Limits of legal liability of the medical laboratory specialist are also appointed by other acts (among others*: the Act on benefits of the health care financed from public means, the Act on healthcare provision, the Act on patient's rights and the Spokesman of patient's rights*) and regulations of the Minister of Health (among others: *Regulation on detailed rules and procedures for disciplinary proceedings in relation to medical laboratory specialists*).

Medical laboratory specialist in relation to a patient

Polish CEMLS emphasizes that „Medical laboratory specialist shall perform his/her functions with respect for a human being" [11], treating the good of a patient as the most important aim of his/her work. In everyday practice the fact of a limited contact with the patient hampers the realization of this demand. Sometimes, meeting of both people takes place at the moment of taking the biological material and/or communicating results of the examination. However, this contact is usually limited to familiarization with personal data of an individual and having a bit of their biological material in a test tube provided by another employee of the Health Service.

In this situation it is easy to lose any personal character of the relation between the medical laboratory specialist and the patient.

Personal reference from the medical laboratory specialist towards the patient is possible thanks to constant awareness that the work carried out is protecting the health and the life of a concrete man. This awareness is expressed through using all of the acquired knowledge and skills in order to obtain credible results. It demands prior reflection on the effectiveness and the usefulness of planned procedures. At the request of the patient the medical laboratory specialist should grant him/her with intelligible information concerning the examination. Further steps are careful collection, archiving, securing and analysis of the biological material. Patient care is also manifested in following professional secrecy. Findings belong to the donor of the sample and can be provided for other people or institutions exclusively with the owner's permission. A serious violation of personal relationship with the patient includes therefore such reprehensible behavior as e.g.: ceasing to perform the commissioned examination, falsifying or withholding the results, making samples or obtained information about patient's condition available to outsiders, improper storage of biological material, etc.

Medical laboratory specialist in relation to his/her own environment

Apart from responsibility for a patient, CEMLS strongly emphasizes that „a medical laboratory specialist shall practice the profession being committed to professional self-governance, development of irreproachable professional attitudes and to continuous professional development" [11]. Dynamic developments in the science, including medical laboratory analysis, demand from the medical laboratory specialist unceasing enhancement of his/her qualifications and obtaining new specializations. What seems essential is participation in scientific conferences, trainings and studying of specialist literature. The medical laboratory specialist should share the acquired knowledge with his/her colleagues. When performing managerial functions he/she cannot hamper their subordinates' efforts to raise their qualifications but rather should motivate them to hone them.

An expression of the medical laboratory specialist's responsibility for their own environment is active participation in initiatives to improve the organization of work and to raise standards of examination quality. It is necessary to respect the principle of acting within one's own competence. In case of any problems exceeding the knowledge of the medical laboratory specialist or doubts concerning acquired results and their interpretation, the medical laboratory specialist should seek advice of appropriate specialists. The next principle, which provides for the accountability for the ethos of the work environment, is a joint responsibility for the performed work and the functioning of the laboratory. The medical laboratory specialist who notices any mistakes in the conduct of a colleague should with due tact turn first to the person concerned and in case of a special situation to his/her superior.

Medical laboratory specialist in relation to the medical environment and society

The medical laboratory specialist enjoys in Poland a status of the profession of public trust [3]. Waszkiewicz stresses that performing that kind of profession involves obtaining information of a private and sometimes intimate nature [23]. The protection of the interest of a person this information concerns demands preservation of secrecy, professionalism and the principles of ethics. Those who execute profession of the public trust are therefore bound by professional secrecy. They are required to have high qualifications, which include, among others, thorough

education, work experience, relevant personality traits and health status. Moreover, high moral requirements, whose outline is included in the code of ethics of a given profession, are put before them. Care to confirm and preserve the prestige of the profession of public trust in relation to the medical personnel of diagnostic laboratories rests with NCLD. In addition to the supervision of members of the self-government body, an expression of this particular concern is the developed code of ethics.

CEMLS reminds of the obligation of every medical laboratory specialist for continuous building of public trust in the medical community and the society as a whole, "which is an indispensable requisite for appropriate performance of the tasks connected with health protection" [11]. Continuous building of this trust includes, but is not limited to, several key actions mentioned in the Code. The first of them is the compliance with the rules of propriety in human relations. In particular, it is about keeping the proper respect for the patient, his family and all the people in the environment. This requirement involves an obligation to co-operate with the doctor commissioning the tests. The harmonious cooperation obviously does not rule out the right of objection of the conscience expressed where justified. In the event of conflict with his/her conscience, the medical laboratory specialist can refuse to perform the examination commissioned onto him, informing the doctor and his/her superiors. The medical laboratory specialist is also able to turn to his/her own corporation for assistance and legal protection, if any forms of pressure from his/her supervisors or other Health Service employees are being exerted on him/her.

A crucial factor in constant building of public trust by medical laboratory specialists is the virtue of honesty, manifested in diverse situations. The first situation mentioned by CEMLS is scientific activity of the laboratory staff. Plagiarism in the scientific work or adapting results of analyses to a thesis put forward previously would be a reprehensible behaviour of the medical laboratory specialist. Also making results of laboratory tests available to unauthorized people, among others to employers and insurance companies would be a sheer dishonesty. Another sphere in which the virtue of honesty comes to the fore is the economy. The medical laboratory specialist cannot make services provided conditional on an extra bonus coming from, e.g. companies representing producers of medical equipment, insurance companies, patients or other people or institutions interested in the test results. In the organization of work and management of the laboratory, the medical laboratory specialist is obliged to make transparent decisions, avoiding unfair competition and nepotism.

CONCLUSIONS

Polish CEMLS is probably the world's first code of ethics of medical laboratory specialists. It harmoniously fits into the set of medical profession codes of ethics and constitutes an important component of professional ethics. Under state law, it appears appropriate to treat it as a "soft law" document. CEMLS constitutes an expression of the formulated ethos of the medical laboratory specialist and a point of reference for representatives of this profession in making the right moral decisions. The code emphasizes that the guiding norm of conduct of the medical laboratory specialist is the good of the human person. The code points out that the service to the patient, building proper relationships at work and strengthening of the public trust enjoyed by medical laboratory specialists are all possible based on the values, i.e. honesty, integrity and competence. The ethos of Polish medical laboratory specialists, of which CEMLS is a synthesis, has its roots in the Hippocratic tradition of medicine. Thus, one should suppose that it possesses a number of elements common with moral

attitudes of medical laboratory specialists from other countries in Europe and the world.

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KLK-targeted therapies for prostate cancer

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ABSTRACT

Alternative treatments are urgently needed for prostate cancer, especially to address the aggressive metastatic castration-resistant disease. Proteolytic enzymes are involved in cancer growth and progression. The prostate produces several proteases, the most abundant ones being two members of the kallikrein-related peptidase (KLK) family, prostate-specific antigen (PSA) and KLK2. Despite the wide use of PSA as a clinical marker, the function(s) of PSA and other KLKs in prostate cancer are poorly known. Hypothetic roles of KLKs in prostate cancer include activities that may both promote and inhibit cancer growth and metastasis, including the antiangiogenic activity of PSA. Thus it may be possible to control prostate cancer growth by modulating the proteolytic activities of KLKs. PSA and KLK2 are especially attractive targets for prostate cancer treatment because of their proposed roles in tumor development and inhibition of angiogenesis in combination with their prostate selective expression. So far the number of molecules affecting selectively the activity of KLKs is limited and none of these are used to treat prostate cancer. Prodrugs that, after cleavage of the peptide part by PSA or KLK2, release active drug molecules, and PSA-targeted therapeutic vaccines have already been tested clinically in humans and the first results have been encouraging. Although KLKs are attractive targets for prostate cancer treatment, much remains to be done before their potential can be fully elucidated. The objective of this review is to address the current state of the KLKs as novel therapeutic targets for prostate cancer treatment.

INTRODUCTION

Prostate cancer is a considerable health care problem. With 900,000 new cases and about 260,000 deaths worldwide in 2008, it is the second most frequently diagnosed cancer and the sixth most common cause of cancer death in men [1]. In the UK, the lifetime risk of developing prostate cancer is estimated to be 1 in 8 (Cancer Research UK). Since the approval of prostate-specific antigen (PSA or kallikrein-related peptidase-3, KLK3) test by FDA in 1986, PSA has been the most widely used cancer marker [2]. However, extensive screening with PSA has lead to detection and unnecessary treatment of cancers that would not have surfaced clinically without screening [2]. Prostate cancer often presents as a multi-focal tumor with various degrees of aggressiveness. Because of the widespread use of screening, most prostate cancers are presently detected at an early stage and have favorable prognosis. While most patients can be cured by radical prostatectomy or radiotherapy, these are associated with side effects, and about one third of the tumors relapse [3]. Patients can be treated by androgen deprivation, but eventually most of them, and 10-20% within 5 years, become resistant to this therapy, i.e., develop castration-resistant prostate cancer (CRPC) [4]. Currently there is no cure for these cancers [5]. While CRPCs respond to some treatment modalities, the effect on survival is generally modest, i.e., some months. Therefore, it is important to develop alternative treatments that are either curative, prevent the development of CRPC and/or formation of metastatic lesions that eventually kill the patients, or to slow down the growth of small tumors, in order to prevent them from surfacing clinically within the lifetime of the patient. Proteolytic enzymes (proteases), including 15 members of the kallikrein-related peptidase (KLK) family, are potential targets for treatment of prostate cancer [6]. Despite the widespread use of PSA as a clinical marker, the function(s) of PSA and other KLKs in prostate cancer are poorly known [7]. While several KLKs may be involved in prostate cancer development, efforts to target them for treatment of prostate cancer have concerned the two major proteases produced in prostate, i.e., PSA and KLK2. Thus this review will focus mainly on these KLKs and their use as novel therapeutic targets for prostate cancer treatment.

PROTEASES IN CANCER

Since the discovery of the role of proteases in food degradation, proteases have been found to be involved in almost all biological pathways and networks, performing several essential functions in all living organisms, from fertilization and development to normal physiology [8,9]. Proteases (also called peptidases or proteolytic enzymes) may exhibit highly selective substrate cleavage or have broader specificity. About 600 human proteases, which are collectively called the degradome, representing ~2 % of the whole human genome are known [10]. Among the serine proteases, KLKs form a family of 15 trypsin- and chymotrypsin-like proteases [11,12]. Protease activity is controlled by several mechanisms, including regulation of gene expression, activation of their inactive pro-forms (zymogens) either autocatalytically or by other proteases, inhibition of their activity by endogenous protease inhibitors, and phosphorylation [8,13]. Many proteases, including those of the KLK family, act in cascades or networks, which facilitates signal amplification and stringent regulation of their activity [14].

Alterations in proteolytic systems underlie several pathological conditions, including cancer, and proteases have been found to play a significant role at virtually all stages of tumor progression [9]. The roles of proteases in cancer have been widely studied since the discovery of their

role in cancer cell invasion, which is a prerequisite for tumor invasion and metastasis formation. In addition to degrading extracellular matrix proteins and adhesion molecules facilitating cell invasion, proteases have several other functions relevant for cancer, including activation of protease-activated receptors (PARs) and regulation of the activity of other signaling molecules, like kinases and growth factors [8,13,15]. Cancer has been thought to be primarily associated with increased proteolytic activity and while this is true for many proteases, some proteases exert opposite effects, such as acting as tumor suppressors by suppressing angiogenesis or inducing apoptosis [16,17].

Expression of KLKs in prostate cancer

The prostate produces several proteases, the most abundant ones being two KLKs, PSA and KLK2 [18]. Shaw and Diamandis reported that all 15 KLKs are expressed in the prostate at the mRNA level [18]. In tissue extracts they found, in addition to KLK2 and PSA, KLK1, -4, -5, -9, -11, -13, -14 and -15. PSA is expressed in differentiated luminal epithelial cells of the prostate and secreted into seminal fluid. The levels in extracellular fluid of the prostate are up to 2 µM or in prostate tissue 10 mg/g of tissue [18,19]. Most of this PSA is enzymatically active [19]. However, when active PSA and other KLKs reach circulation, they are rapidly inactivated by protease inhibitors that are present in vast excess in circulation. While PSA is a major constituent of seminal fluid, only a minor part of it leaks out into circulation. Interestingly, the tissue concentrations of PSA are lower in malignant than in normal prostatic epithelium and they are further reduced in poorly differentiated (high Gleason grade) tumors [20]. In spite of this, PSA is the best cancer marker presently available [2,7]. This is based on increased leakage into circulation from malignant prostatic tissue that has lost connection with the prostatic ducts [21]. The clinical use of PSA determinations has been reviewed in several recent articles [22,23].

Among the KLKs, KLK2 has attracted most interest after PSA, due to its prostate specificity and relatively high expression levels [18]. Contrary to PSA, KLK2 expression in prostate cancer is higher than in benign prostate [23,24]. The ratio between hK2 and PSA mRNA increases with increasing grade [25].

Noteworthy, single nucleotide polymorphisms (SNPs) in KLK genes have been shown to be associated with prostate cancer [26]. Some of these SNPs affect the expression levels of KLKs. However, apart from these genetic polymorphisms and hormonal regulation [11], the mechanisms behind the altered regulation of KLK expression in tumors still remains largely unsolved.

Functions of KLKs in prostate cancer

The suggested functions of KLKs include both those that promote and inhibit tumor growth and metastasis [7,11,12,27]. The physiological function of PSA, and perhaps also other prostatic KLKs, is to promote sperm motility by dissolving the seminal clot formed after ejaculation by cleaving semenogelins. KLKs are also able to cleave several prostate cancer related substrates, at least *in vitro* [11]. However, hypothetic functions based on *in vitro* cleavage should be interpreted with caution. In clinical studies, low PSA levels in prostate cancer tissue are associated with poor prognosis [20,28], while high PSA levels are associated with low blood vessel density [29,30]. However, the PSA concentrations in serum are sometimes increased decades before the development of otherwise detectable tumors [31,32]. This suggests that PSA may initiate or facilitate early cancer development.

Cancer cells have to acquire several biological capabilities during the multistep development of tumors described by Hanahan and Weinberg

in *Hallmarks of Cancer* [33]. Several described or hypothesized functions of KLKs are relevant for these effects [7]. The ability to proliferate and evade growth-suppressing signals is one of the essential properties of cancer cells. Several studies suggest that PSA and other KLKs may promote the growth of prostate cancer by stimulating cell proliferation [34,35]. Furthermore, PSA has been found to promote the growth of prostate cancer xenograft tumors [34]. In contrast to these studies, Bindukumar *et al.* [37] found that subcutaneously administered PSA reduced the growth of xenograft tumors in mice. Several KLKs have been found to activate growth-factors and PARs [11,15], which lead to a wide array of responses, including promotion of cancer cell growth and invasion. In addition to increasing cell proliferation, PSA has been shown to reduce apoptosis [35], which is also essential for cancer development.

Like all tissues, tumor needs nutrients and oxygen and ability to remove waste and carbon dioxide in order to grow and survive [33]. This requires vascularization and thus tumors need to develop new blood vessels in order to grow beyond a size of 2-3 mm^3 [37]. Prostate cancer grows unusually slowly after reaching this size, which corresponds to the time when it can be detected by prostate biopsy of men with elevated serum concentrations of PSA [31]. The slow growth of prostate cancer could be dependent on the antiangiogenic activity of PSA. Several studies have addressed the antiangiogenic role of PSA, which has been demonstrated in cell culture models at sub-physiological PSA concentrations [16,38,39]. In a pioneering study by Fortier *et al.*, PSA was shown to inhibit endothelial cell tube formation, growth, invasion and migration [16]. They further showed that subcutaneous administration of PSA inhibits angiogenesis in an *in vivo* model of blood vessel growth [38]. The mechanism by which PSA exerts its antiangiogenic effect is unclear. Even the dependence on enzymatic activity is controversial [38]. However, our studies strongly suggest that PSA activity is needed for the antiangiogenic activity, as the enzymatic activity of different PSA forms present in seminal fluid correlates with the antiangiogenic activity [39]. Furthermore, inhibition of PSA by small molecule inhibitors or an antibody abolishes the antiangiogenic activity [40], while the stimulation of PSA activity by peptides enhanced it [41].

Several KLKs, like PSA and KLK2, are able to degrade extracellular matrix proteins and activate other extracellular matrix degrading proteases or inactivate their inhibitors [11,14]. These studies suggest that KLKs are involved in proteolytic cascades facilitating prostate cancer growth and metastasis [14]. Indeed, PSA-treatment has been found to increase invasion of prostate cancer cells *in vitro* [42]. Other studies suggest that PSA may play a role in the development of bone metastases (reviewed in [11,43]).

Knockout studies of PSA or KLK2 have not been performed as mice and other animals used for such studies do not have genes encoding PSA or KLK2 [11]. Most studies aiming to solve the functions of KLK2, PSA, and other KLKs have utilized cancer cell lines. However, the *in vitro* growth characteristics of these cells may not necessarily predict tumorigenicity and different cell lines may show very different responses [7]. Furthermore, cancer cells grown in an isolated environment behave very differently from those in tumors and in contact with extracellular matrix and stromal cells [44]. Transgenic mice expressing PSA and/or KLK2 in the prostate have been developed. In these, neither PSA nor KLK2 have been found to initiate cancer or cause any morphological changes [45]. However, the PSA levels in these are about 1000-fold lower than those in the human prostate.

Taken together, these studies suggest that PSA and other KLKs may affect tumor growth and

perhaps even initiate cancer development. The effects of KLKs may be different at different stages of tumor growth, e.g., PSA may favor tumor development at early stages of cancer, for example by activating growth factors, but at later stages it may inhibit tumor growth by its antiangiogenic activity [7].

KLK-TARGETED THERAPIES FOR PROSTATE CANCER

Recently, proteases have been estimated to represent 5-10% of the potential drug targets [46,47] but the number of new approved protease inhibitors is still limited [48]. A problem with proteases as drug targets is the lack of specificity. Thus, inhibitors tend to react with similar proteases affecting a broad range of protease activities that are crucial for normal physiology. For example, lack of specificity was a major reasons for the failure of early matrix metalloprotease (MMP)-inhibitors, as they also inhibited MMPs that are needed for normal tissue function, or act as tumor suppressors [46,47,17].

PSA and KLK2 are attractive targets for prostate cancer treatment because of their possible roles in tumor development, metastasis and inhibition of angiogenesis, and their prostate selective expression, which together with the lack of active forms in circulation makes systemic effects unlikely. Furthermore, as their proposed physiological function is related to liquefaction of the seminal fluid clot, their targeting is not likely to cause severe side-effects other than those related to fertility. In addition to PSA and KLK2, KLK4 is also a potential target for prostate cancer treatment [49]. KLK4 is overexpressed in prostate cancer, promotes cell proliferation and cleaves several cancer associated substrates, including PARs [11,50,51].

Several naturally occurring proteins, including serpins, Kazal-type serine protease inhibitors and α_2-macroglobulin, inhibit KLKs [49,52]. However, these are rather non-specific inhibitors. So far the number of molecules affecting selectively the activity of individual KLKs is very limited and none of these are used to treat prostate cancer [6,49,52]. For PSA, both activity-stimulating and inhibiting compounds have been developed, while for KLK2 and KLK4 only inhibitors have been described (Table). Prodrugs that are activated by PSA or KLK2, and PSA-targeted therapeutic vaccines have already been tested clinically in humans and preliminary results are encouraging [53,54] (Table).

PSA inhibitors and stimulators

Several peptide-based or small molecule inhibitors for PSA have been described (reviewed in [6,27,49,52]). These have been found either by high-throughput screening or by rational drug design. Several antibodies that inhibit or stimulate PSA activity have also been described [55]. Some of these inhibitors have been tested in prostate cancer relevant models and found to inhibit antiangiogenic activity of PSA in a cell culture model [40], inhibit the growth of prostate cancer cell lines or exert a small inhibitory effect on xenograft tumor growth [56]. It should be noted that the specificity of these inhibitors has not been thoroughly characterized.

Since PSA shows antiangiogenic activity and, thus could inhibit development of prostatic tumors, we have been interested in molecules that stimulate the activity of PSA. By screening of almost 50,000 drug-like small molecules, we found some PSA inhibitors but did not identify compounds that stimulate PSA activity [40]. While small molecule drugs have several advantages as compared to peptides, their specificity for similar proteases is more limited. Furthermore, only 30-50% of the targets that represent an opportunity for therapeutic intervention have been suggested to be amenable to traditional small molecule approaches [57]. Thus, we have used phage-display to develop peptides,

Table **Summary of the major findings on different KLK targeted therapies for prostate cancer**

Strategy/target	Agent	Major outcome/therapeutic effect[a]	References
Inhibitors			
PSA	small molecules or peptide based	Inhibit prostate cancer cell and xenograft tumor growth; inhibit antiangiogenic activity of PSA	6,27,40,49, 52,56
KLK2	modifed serpin[b], peptide	Reduce xenograft tumor growth[b]	63-66
KLK4	peptide	Not determined	68,69
Stimulators			
PSA	peptide, small molecule	Stimulate antiangiogenic activity of PSA in cell model	58-62
Prodrugs			
PSA	PSA substrate combined with a toxic drug molecule	Selectively kills PSA-producing cells in vitro; selective antitumor effect on PSA-producing tumor xenografts in mice and monkeys; significant improvement of symptoms in patients with benign prostatic hyperplasia with only mild, locally limited side effects	73-84
KLK2	KLK2 substrate combined with a toxic drug molecule	Significant antitumor effect in tumor xenografts in vivo, but prolonged administration caused local toxic effect; less effective than similar PSA-activated prodrug	85
Vaccination			
PSA	antigen (PSA), DNA-based vaccines, usually include other antigens	Safe in phase I and II studies, showing prostate specific T lymphocyte responses and benefit for some of the patients	53-54

[a] The mentioned outcomes and therapeutic effects may be valid only for some of the agents for a given strategy and target.

[b] The agent, modified serpin, not specific for KLK2.

which stimulate the activity of PSA several fold at µM concentrations [58,59]. These peptides also stimulate PSA-activity towards protein substrates but they do not affect the activity of several enzymatically or structurally related proteases (Mattsson et al., unpublished data) [59]. The use of peptides for drug discovery is a rapidly emerging field. The pharmacokinetic and other properties of peptides can be modified and they can serve as starting structures for development of peptidomimetics. We have been able to significantly improve the stability of some peptides stimulating PSA activity and created the first pseudopeptides, in which parts of the peptide have been replaced by non-peptidic structures without loss of bioactivity [60,61]. We hypothesize that modified peptides or peptidomimetic compounds based on these can be used for imaging and proof of principle studies, and eventually for treatment of prostate cancer. The peptides enhance the antiangiogenic activity of PSA in cell culture models [41] but in preliminary animal studies, the first generation peptides have not shown any major effect on tumor growth (our unpublished results). This is not surprising as the peptides are quickly excreted. Using pharmacophore-based virtual screening we have recently identified the first small drug-like molecule that stimulates PSA activity [62].

KLK2 and KLK4 inhibitors

Since many cancers, including prostate cancer, are associated with increased activity of several proteases, inhibitors, in addition to those for PSA, have been developed for KLK2 and KLK4 for the targeting of prostate cancer. Cloutier *et al.* have used phage-display to screen a library of variants of α_1-anti-chymotrypsin (ACT), which inhibits several proteases. They identified a modified version of ACT that showed selectivity towards KLK2 [63], but it was later found to also inhibit several other KLKs, especially KLK4, KLK5 and KLK14 [64]. This molecule, called MD-PK67b, has been shown to reduce the growth of prostate cancer xenograft tumors producing KLK2. Further clinical studies, including evaluation of safety in humans, have been initiated [64].

As our phage-display approach was successful with PSA, we also developed peptide inhibitors for KLK2 using this approach [65]. While all of the PSA-stimulating peptides were cyclic containing one or two disulfide-bridges, all the KLK2 inhibiting peptides were identified in linear peptide libraries. The identified peptides inhibited KLK2 at µM concentrations. Like with PSA-stimulating peptides, we have been able to significantly improve the stability of the KLK2-inhibitory peptides [66].

Sunflower trypsin inhibitor (SFTI), which is a 14 amino acid residue cyclic peptide structurally similar to Bowman-Birk family of serine protease inhibitors, is a potent and broad-range protease inhibitor [49,67]. Recently, SFTI has been modified to selectively and efficiently inhibit KLK4, using a combination of molecular modeling and substrate screening [68], and further *in silico* screening of inhibitor variants in complex with KLK4 or trypsin [69]. Although promising results with the SFTI-based KLK4 inhibitor have been obtained using ovarian cancer models [70], its effect on prostate cancer have not yet been reported.

PSA- and KLK2-activated prodrugs

Perhaps the most promising results concerning the use of KLKs in prostate cancer treatment have been obtained using prodrugs that are activated by PSA or KLK2. Protease-activated prodrugs are promising for targeted delivery of drugs into a specific tissue. The inactive prodrug consists of a toxic drug molecule conjugated to a peptide. The prodrug is activated in the target tissue through cleavage of the peptide moiety

by a specific protease leading to release of the active drug molecule [71,72]. Thus, the effect of the drug is directed to a specific tissue. PSA and KLK2 are well suited as prodrug activators as they have highly tissue specific expression, i.e., significant amounts of active PSA and KLK2 are found only in the prostate [6,18].

Several PSA-activated prodrugs have been developed using peptide sequences that are highly selective for PSA [73,74]. Drug molecules conjugated to these peptides include doxorubicin [73-75], vinblastine [76], 5-fluorodeoxyuridine [77], thapsigargin [78] and paclitaxel [79]. A doxorubicin conjugated prodrug L-377,202 selectively killed PSA-producing human prostate cancer cells *in vitro* and was 15 times more effective than conventional doxorubicin at inhibiting the growth of human prostate tumor xenografts *in vivo* [74]. Another PSA-activated prodrug, PRX302, with the bacterial toxin precursor proaerolysin, showed selective antitumor effect on PSA-producing tumor xenografts in mice, caused extensive damage to PSA-producing prostate cells in monkeys and showed no toxicity in other tissues [80]. These two PSA-activated prodrugs have been taken to phase I and II clinical trials, where both were well tolerated, although L-377,202 caused neutropenia at higher doses [75]. Intraprostatic injections of PRX302 lead to significant improvement of symptoms in patients with benign prostatic hyperplasia while only mild, locally limited side effects were reported [81].

Recent studies of PSA-activated prodrugs report utilization of cell penetrating peptides to deliver drugs inside prostate cancer cells [82], development of prodrug modifications using e.g. albumin as a drug carrier [83] and synthesis of fusion peptides with multiple specificities to target the drug effect to the cells that express specific receptors [84].

A thapsigargin-based prodrug has been developed for KLK2 [85]. A prodrug with a KLK2 peptide substrate conjugated to the thapsigargin analog, L12ADT, showed a significant antitumor effect in human prostate tumor xenografts *in vivo*, but prolonged administration caused local toxic effects [85]. Moreover, the antitumor effect was only modest when compared to a similar PSA prodrug with thapsigargin.

PSA-targeted therapeutic vaccines

Prostate cancer is considered an attractive target for development of therapeutic vaccines as it expresses several prostate-specific proteins and generally grows very slowly. Indeed, several vaccination strategies have been established, some of which target PSA (reviewed in [53,54]). Usually these vaccines also target other antigens in order to improve the immune response. For PSA, viral and DNA-based vaccines have been used, both encoding PSA and, usually, other antigens. These vaccines have been found to be safe in phase I and II studies, showing prostate specific T lymphocyte responses and, at least in some cases, some benefit for the patients [53,54]. It seems that these vaccines would be especially beneficial for patients with early-stage disease but results from phase III studies are not available yet.

CONCLUSIONS

PSA is an established marker for prostate cancer and several other KLKs are potential markers. As circulating PSA level is often used as a surrogate marker for tumor burden in preclinical and clinical studies [73-76,80,83], and more importantly to monitor relapse in patients, it would be important to address whether KLK targeted therapies could affect PSA levels also otherwise than by affecting the volume of the PSA producing tumor tissue. This is possible as PSA-targeted activity modulators and vaccination may affect, through several mechanisms,

the circulating PSA levels and also the detection of PSA by immunoassay. While in prodrug studies PSA seems to be a good marker for tumor burden, the therapy has also been found to induce initial leakage of PSA into circulation [81], perhaps because of tissue destruction.

While conclusive evidence for the roles of the KLKs in prostate cancer development is still lacking, KLKs expressed in the prostate are likely to have functional role(s) making them potential targets for treatment of prostate cancer. We have proposed that PSA promotes the growth of small tumors, but may inhibit development of large tumors at the stage when new blood vessels are needed [7]. In addition to PSA and KLK2, some other KLKs, like KLK4, are also potential targets for prostate cancer treatment. However, these KLKs have been less studied and their expression is not as restricted to the prostate as that of KLK2 and PSA. Therefore development of treatment based on these is more challenging. While several inhibitors for PSA and KLK2, and stimulators for PSA have been developed, their efficacy has not yet been tested in higher primates or humans. Some of these compounds have been promising in cell culture and mouse xenograft tumor models. However, the models used so far have several limitations and do not reflect the complexity of human prostate cancer. Furthermore, the specificity of the compounds has not been fully elucidated. Prodrugs that are activated by PSA or KLK2 have been tested clinically in humans with encouraging preliminary results. It is foreseen that these prodrugs, along with vaccines targeting PSA, may be the first KLK-based treatment modalities for prostate cancer. In conclusion, KLKs are attractive targets for prostate cancer treatment, but much remains to be done before their potential can be fully harnessed to treat prostate cancer.

DISCLOSURES

Ulf-Håkan Stenman holds patents for KLK-activity modulating peptides. Hannu Koistinen and Johanna Mattsson have nothing to disclose.

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