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

Prostate cancer genetics: a review

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ABSTRACT

Over the past decades, research has focussed on identifying the genetic underpinnings of prostate cancer. It has been recognized that a number of forms of genetic changes coupled with epigenetic and gene expression changes can increase the prediction to develop prostate cancer. This review outlines the role of somatic copy number alterations (SCNAs), structural rearrangements, point mutations, and single nucleotide polymorphisms (SNPs) as well as miRNAs. Identifying relevant genetic changes offers the ability to develop novel biomarkers to allow early and accurate detection of prostate cancer as well as provide risk stratification of patients following their diagnosis. The concept of personalized or individualized medicine has gained significant attention. Therefore, a better understanding of the genetic and metabolic pathways underlying prostate cancer development offers the opportunity to explore new therapeutic interventions with the possibility of offering patient-specific targeted therapy.

1. INTRODUCTION

Patients with clinically localized prostate cancer experience a phenotypically wide spectrum of natural history ranging from indolent tumors which will never require treatment to highly aggressive, metastatic and ultimately fatal cancer. Currently, we have limited tools to risk stratify patients with prostate cancer – stage, grade and PSA. Although histologic grade provides an important predictor of tumor biology, patients with the same Gleason score can experience widely different outcomes.

It has been recognized that some patients with prostate cancer have a hereditary basis to their disease. This has led to definitions of both "familial" and "hereditary" prostate cancer to distinguish them from the more common "sporadic" tumors. Familial prostate cancer is defined as having at least one first degree relative with prostate cancer[1]. Hereditary prostate cancer is defined as a family with three affected generations, three first-degree relatives affected, or two relatives affected before age 55 years[2]. In addition, prostate cancer incidence also varies based on ethnicity and environmental factors that will be discussed further.

Patients may experience significant morbidity and loss of quality of life from the overtreatment of clinically indolent prostate cancers[3]. However, many patients continue to die of local advanced and metastatic prostate cancer. The Canadian Cancer Society estimates that 23,600 men will be diagnosed with prostate cancer in 2013 and that 3,900 will die of the disease[4]. Therefore, a better understanding of the genetic and molecular characteristics distinguishing indolent from lethal prostate cancers is necessary in order to better manage patients and provide the appropriate treatment to the appropriate patient at the appropriate time.

Over the past twenty years, the scientific community has come to believe that carcinogenesis is the result of genetic and/or epigenetic changes to protein-coding oncogenes and tumor suppressor genes. In the case of solid-organ malignancies such as prostate cancer, these result typically from somatic genetic events. However, in addition to these somatic genetic changes, it has also become clear that many cancers, including prostate, exhibit loss of function of tumor suppressor genes due to epigenetic changes in expression. Epigenetic mechanisms include biochemical modification of histones supporting DNA, modification of the DNA itself and expression of non-coding RNAs, including miRNAs.

Despite the high prevalence of prostate cancer, little is known about its cause. Many genes have been implicated in the development of both sporadic and particularly hereditary prostate. Unfortunately, attempts at identifying a reliable biomarker have thus far proved unsuccessful due in large part to the highly variable disease, multiple implicated epidemiological factors and advanced patient age at diagnosis.

2. ENVIRONMENTAL FACTORS

In addition to individual clinical heterogeneity, prostate cancer has highly variable incidence rates depending on race, geographic location and modifiable environmental factors[5].

Studies have found that incidence rates of prostate cancer vary depending on geographic location, even amongst the same ethnic group[5]. The lowest rates of clinical prostate cancer are found in Asian populations[6], intermediate rates in Hispanic and Caucasian populations[7] and the highest rates in African American populations[7]. Even within Europe, higher rates are seen in Northern Scandinavian populations and lower rates are seen in Mediterranean populations[8,9]. This suggests that environmental and as yet uncharacterized epigenetic changes likely play a significant role in prostate cancer.

3. GENETICS OF PROSTATE CANCER

Genetic and epigenetic changes occur at many levels. Genetic alterations have offered use as biomarkers, particularly in the case of breast and ovarian cancer. Mutations in BRCA1 and 2 have been found to confer a high risk of the development of these diseases[10]. Similar work is underway in both bladder and colorectal cancer.

Current genes of interest as biomarkers for prostate cancer include RNase L (HPC1, 1q22), MSR1 (8p), ELAC2/HPC2 (17p11). These genes have been identified as hereditary tumor suppressor genes in prostate cancer.

Genetic changes involved in carcinogenesis may be present either in the host germline DNA or isolated to the tumor genome. Prostate cancer is known to have an extraordinarily complex genetic makeup including somatic copy number alterations, point mutations, structural rearrangements and changes in chromosomal number (Table 1)[11].

3.1 Somatic copy number alteration

Somatic copy number alterations (SCNAs) are gains or losses in genetic material that affect a larger fraction of the cancer genome than do any other form of somatic genetic alteration[12]. They have an integral role in both the activation of oncogenes and the inactivation of tumor suppressor genes. SCNAs are found in nearly 90% of prostate tumors[11]. In the primary lesion, these tend to be small, focal changes whereas in metastatic tumors, hundreds of aberrations can be found affecting a large portion of the genome. This may reflect increasing genomic instability with disease progression. Beroukhim found that prostate cancer exhibited more SCNAs than most of the other 26 types of cancer examined[13].

Primary tumors frequently exhibit deletions on chromosome 6q, 8p, 10q, 13q and include genes including NKX3-1, PTEN, BRCA2 and RB1. Conversely, castrate-resistant metastatic tumors often exhibit amplification of chromosomes X, 7, 8q, and 9q and include genes from the androgen receptor pathway and the MYC oncogene.

The clinical utility of SCNA has been limited due to difficulty in detection. CT-guided prostate biopsy has yielded success of only 60-70%. Therefore, interest has risen in their identification in blood and bone marrow in the form of circulating and disseminated tumor cells[11].

3.2 Structural rearrangements

As DNA unwinds during replication and transcriptions, double-stranded breaks may occur. Improper repair of these can result in both intra- and inter-chromosome rearrangements. TMPRSS2:ERG is perhaps the best studied of these in prostate cancer. This rearrangement occurs in nearly 50% of all primary prostate tumors. Functionally, this places the growth-promoting activity of the ERG oncogene under the control of the regulatory elements of the androgen-responsive TMPRSS2 gene[14]. Nam et al. showed that expression of this gene fusion confers an increased risk of disease relapse after treatment for clinically localized prostate cancer (HR 7.1, 95% CI 1.1-45)[15].

A number of other rearrangements have been described in prostate cancer including ESRP1:CRAF, the ETS family and RAF kinase gene fusions[16-20].

Though there is not a direct relationship between ERG rearrangement and SCNAs, ERG rearrangement has been associated with 10q, 17p and 3p14 deletions[21]. On the other hand, those tumors without ERG rearrangement exhibit 6q and 16q deletion and 7q amplification[21].

Paired-end whole-genome sequencing of patients with high-risk primary prostate tumors showed a median of 90 structural rearrangements per tumor genome, highlighting the

Table 1 Genetic changes associated with prostate cancer tumorigenesis

Genetic change	Description	Mechanism	Example
Somatic copy number alterations (SCNAs)	Gain or loss in genetic material	Role in both oncogenic activation and tumor suppressor inactivation	Deletions on chromosome 10q leads to PTEN LOF[11]
Structural rearrangements	Improper repair of DNA breaks leads to intra- and inter-chromosome rearrangement	Rearrangements place otherwise unrelated genes in juxtaposition	Fusion of TMPRSS2:ERG results in oncogenic activation of ERG under the control of the TMPRSS2 androgen-response element[14]
Point mutations	Changes in specific nucleotides or amino acids resulting in altered gene products	Nucleotide changes result in proteins with altered function or stability	HOXB13 G84E variant confers an elevated risk of prostate cancer, specifically early-onset or hereditary through regulation of transcription of AR target genes[46-49]
Single nucleotide polymorphisms (SNPs)	Variation in a single nucleotide differing between individuals or chromosomes	SNPs act as markers in gene-mapping. When occurring within a gene, SNPs may directly affect gene function	SNPs in MSMB have been shown to affect the expression of NCOA4 which is an AR co-activator[61]
miRNA	Small, non-coding RNA molecules which modulate mRNA expression	The majority result in down-regulation though a few cause up-regulation or destruction of the target mRNA	MiR-21 targets PDCD4 and PTEN mRNAs and causes decreased apoptosis[80]

PTEN: phosphatase and tensin homolog; LOF: loss of function; TMPRSS2: transmembrane protease, serine 2; ERG: ETS-related gene; HOXB13: homeobox 13; AR: androgen receptor; MSMB: beta-micro-seminoprotein; PDCD4: programmed cell death 4.

prevalence and complexity of these changes as well as the importance of chromatin structure. Further, in those tumors with TMPRSS2:ERG rearrangement, breakpoints were precise and located in transcriptionally active chromatin that were enriched with transcription factors associated androgen-regulated transcription regions.

3.3 Point mutations

Mutation rate is a key factor in determining a somatic cells risk of malignant transformation. Prostate cancer has a somatic mutation rate between $1x10^{-6}$ and $2x10^{-6}$ which is similar to breast, renal and ovarian cancer[22-24]. With such a rate, each prostate tumor gene may have

many thousand mutations although less than 20 are likely to affect protein stability or function. Mutation of the DNA mismatch repair enzyme MSH6 may result in up to 25-fold more mutations than expected in prostate cancer. Mutations in both tumor suppressor and oncogenes have been described in prostate cancer including TP53, PTEN, RB1 and PIK3CA and KRAS and BRAF, respectively. Further mutations in androgen receptor function, chromatin modification and transcription have also been described.

3.3.1 HPC1 or RNase L gene

The RNase L gene encodes an endoribonuclease and acts in the 2-5-A system which is enzymatically involved in interferon activity. The enzyme is part of the antiviral activity of interferons and is involved in innate immunity via degradation of viral and cellular RNAs. Pertinent to its role in prostate cancer, RNase L has been found to play an important role as a tumor suppressor gene[25].

Smith et al. identified chromosomal region 1q24-25 as a susceptibility locus for familial prostate cancer in 1996[26]. Since this time, there have been numerous studies evaluating many variant mutations as they relate to familial or sporadic prostate cancers with varying results[27,28]. Three missense mutations (Arg426Gln, Asp541Glu, and Ile97Leu) have been primarily implicated in prostate cancer. The R426Q mutation has been associated with increased risk of prostate cancer in Finns, American Caucasians and Japanese[27,29,30]. In a Spanish population, mutations in Arg426Gln were associated with worse prognosis[31]. However, other studies have shown no association between this mutation and sporadic prostate cancer in Swedish and German populations[29,32].

Functionally, *in vitro* studies have shown that the Arg426Gln mutation decreases the enzymatic activity of RNase L thus decreasing tumor suppressor activity and allowing tumor cells to escape apoptosis.

The Asp541Glu mutation has been found to increase the risk of prostate cancer in some Japanese men[33] though this has not been corroborated in European studies[30,34,35]. Studies into the Ile97Leu mutation have not shown a clear correlation with an increased risk of prostate cancer[36].

3.3.2 HPC2 or ELAC2 gene

HPC2 (hereditary prostate cancer gene 2) or ELAC2 (elaC homolog 2) is located on chromosome 17p. It encodes a protein which resembles a family of DNA cross-link repair enzymes. This enzyme is involved in tRNA biosynthesis which removes the 3' trailer from precursor tRNA[37].

As with HPC1, the primary mutations implicated in prostate cancer are missense mutations including Ser217Leu, Ala541Thr, Arg781His, 1641incG, and Glu622Val[38]. The first three of these mutant forms putatively do not affect enzyme/substrate complex formation, cleavage, or substrate release[37]. The 1641incG mutation encodes a non-functional protein[37]. The Glu622Val mutation is proposed to affect the enzymatic function in an unknown fashion[39].

There have been conflicting results with respect to the role of this gene in prostate cancer.

Xu et al. found the Ser217Leu mutation to be related to an increased risk of prostate cancer in both Asian and European populations while the Ala541Thr mutation was associated with an increased risk of prostate cancer in Asian populations[40]. However, other studies have not found similar results[41,42].

3.3.3 MSR1 gene

Located on chromosome 8p22, the MSR1 gene encodes the macrophage scavenger receptors type A. Linkage studies have implicated this gene in a number of diseases including

prostate cancer[43]. A number of mutations have been described including Arg293X, Asp175Tyr, His441Arg, Val113Ala, and Ile54Val. The first of these, Arg293X, was first described in the context of familial prostate cancer amongst patients of European descent while Asp175Tyr was found in an African-American population[44]. Little is known about the final three mutations mentioned above. In addition, the portion of the 3' region of the gene has been linked to an increased risk of prostate cancer in Caucasians[45].

3.3.4 HOXB13

HOXB13 encodes the transcription factor homeobox 13 and is found on chromosome 17q21-22. After linkage studies identified this region as a likely location for genes predisposing to prostate cancer, Ewing et al. screened over 200 genes and found that the HOXB13 G84E variant conferred a significantly increased risk of prostate cancer (OR 20.1, 95% CI 3.5-803.3)[46]. Subsequently, Akbari et al. examined the association between the germline G84E mutation and the risk of diagnosing prostate cancer in a population undergoing prostate biopsy due to either elevated PSA or abnormal digital rectal examination[47]. They found that the mutation conferred a significantly increased risk of prostate cancer amongst white subjects and particularly those with early onset disease (<55 years) or positive family history [OR 5.8 (95% CI 1.3-26.5) and 14.1 (95% CI 2.8-70.3), respectively].

Karlsson corroborated this finding in two Swedish populations[48]. They found the strongest association was in young-onset and hereditary prostate cancer (OR 8.6 and 6.6, respectively). In a large number of prostate cancer families enrolled in the International Consortium for Prostate Cancer Genetics, Xu et al. confirmed this mutation to be unique to European patients[49]. Even within carrier families, the mutation was much more common in those diagnosed with prostate cancer than those not diagnosed (OR 4.42, 95% CI 2.56-7.64). Clinically, they found that carriers of the mutation demonstrated high-risk disease features.

Functionally, this protein regulates the transcription of androgen receptor target genes that have been implicated in prostate cancer development and growth.

3.3.5 SPOP

A new subtype of prostate cancer has been defined by SPOP mutations which are found in up to 13% of primary prostate tumors[50]. These mutations are found in evolutionarily conserved regions of the substrate binding region of the E3-ubiquitin ligase subunit. They were found more commonly in tumors with somatic deletions of 5q21 and 6q21 which encode genes including CHD1, an enzyme involved in chromatin-modification; PRDM1, a tumor suppressor; and FOXO3, a transcription factor. They have also been found to influence the stability of the SRC3/NCOA3 protein and affect androgen-receptor signalling. They have not however shown evidence of ETS rearrangement or mutation in TP53, PTEN or PIK3CA. Thus, this may represent a distinct molecular subtype of prostate cancer.

3.4 Single nucleotide polymorphisms (SNPs)

Genome-wide association studies (GWAS) have detected a wide variety of susceptibility loci (single-nucleotide polymorphisms) that have been implicated in prostate cancer. These studies have typically been undertaken in European populations and in cases of sporadic prostate cancer.

The first GWAS in prostate cancer was published in 2007 and since that time more than 20 GWAS have identified over fifty genetic variants associated with prostate cancer. The majority of these lie on chromosomes 8q24, 3, 17, 22 and X[51,52]. For the most part, the implicated SNPs are found

in intergenic regions and, as a result, many have no putative function.

3.4.1 8q24

The relationship between chromosome region 8q24 and prostate cancer was first identified in 2007 in an Icelandic population. Further studies confirmed that this region has a significant association for men of African-American descent as well as in men with hereditary or familial prostate cancer. Since 2007, several SNPs in this region have been reported to have an association with prostate cancer[53]. This genetic region was originally to be considered non-coding with little or no transcriptional activity and no genes. However, more recent evidence suggests that POU5F1P1, found in this region, encodes a protein involved in carcinogenesis as a weak transcriptional activator[54]. Furthermore, a number of authors have shown that 8q24 encodes enhancers of the proto-oncogene MYC which is located downstream thus suggesting that the associated regions may be involved in MYC regulation[55]. Two polymorphisms in this region, rs4242382 and rs6983267, were found to be associated with metastatic prostate cancer[56].

3.4.2 MSMB

The beta-micro-seminoprotein (MSMB) promoter has been found in a number of GWAS to be associated with prostate cancer risk[57-60]. Variations in this allele have been found to affect the expression of PSP94 and mRNA expression of NCOA4, a nearby gene[61]. Functionally, NCOA4 encodes a protein which interacts with the androgen receptor as a co-activator, enhancing AR transcriptional activity. Lou et al. (2012) reported that the MSMB promoter regulates expression of MSMB-NCOA4 co-transcripts[62]. Chang et al. (2009) found that, functionally, the T allele of this SNP conveyed a higher risk of prostate cancer and have much lower promoter activity than the C allele[63]. Furthermore, treatment with synthetic androgen resulted in a dose-dependent increase of the promoter activity of the C allele, but not the T allele. Ahn et al. found that this allele conveyed an increased risk (RR = 1.24) of metastatic prostate cancer in the Cancer Genetic Markers of Susceptibility (CGEMS) database[56].

3.4.3 KLK2-3

In 2008, Eeles found that a SNP (rs2735839) located between the KLK2 and KLK3 genes was associated with prostate cancer[57]. KLK3 encodes PSA, which has been widely used in prostate cancer screening and diagnosis. Multiples SNPs in this region have been associated with PSA concentration and prostate cancer risk[61]. KLK2 encodes kallikrein-related peptidase 2 which has also been investigated in the evaluation of patients with elevated PSA[65].

3.4.4 HNF1B

Two SNPs on 17q12 were found to be associated with prostate cancer risk[60]. These two SNPs are found on introns of HNF1B, a transcription factor (TCF2). Further studies showed that ten unique SNPs on HNF1B were significantly related to the risk of prostate cancer[53]. SNPs in this region are also associated with diabetes so the possibility that the prostate cancer-SNP relationship is mediated by diabetes must be considered.

3.4.5 JAZF1

JAZF1 (juxtaposed with another zinc finger 1) is located on chromosome 7p15.2. An SNP within intron 2 of this gene has shown an association with the overall risk of prostate cancer and aggressive prostate cancer[58]. A particular SNP, rs10486567 has been found to be associated with biochemical recurrence and castrate-resistance in Ashkenazi Jews[66]. Further GWASs have shown that this locus is also associated with type-2 diabetes and height suggesting

that it may play a role in growth regulation and metabolism.

The JAZF1 gene product appears to act as a transcriptional repressor of NR2C2, a nuclear orphan receptor expressed in prostate cancer[58]. However, functional studies have yet to elucidate the role of either JAZF1 or its SNPs in prostate cancer carcinogenesis.

3.4.6 LILRA3

In a Chinese population, Xu et al. found an SNP on 19q13.4 which is associated with a germline deletion affecting leukocyte immunoglobulin-like receptor A3 (LILRA3)[67]. This is a gene which has previously been implicated in psoriasis and multiple sclerosis but only recently in cancer risk. However, given the role of inflammation in carcinogenesis, this is a potentially fruitful path.

3.4.7 10q26

In a GWAS of a sample of patients in a prostate cancer screening program, Nam et al. found 3 unique SNPs in this region which were associated with aggressive prostate cancer[68]. This study is of particular value as the control patients were derived from the same patient population and had negative biopsies. The three SNPs in this region are found in the vicinity of two genes which have been implicated in the glioblastoma and breast cancer, but not previously in prostate cancer.

3.4.8 15q21

In the same study discussed above, Nam et al. identified 2 distinct SNPs in the 15q21 region which were associated with biologically aggressive prostate cancer[68]. A nearby gene, GATM, encodes a mitochondrial enzyme.

Clearly, many more SNPs have been described; however, to exhaustively review these goes well beyond the scope of this paper.

4. MiRNA

MicroRNAs (miRNAs) are a class of small non-coding RNA which bind to messenger RNA (mRNA) in a manner to modulate mRNA expression. The 5' end of the miRNA binds via a targeting "seed" region to a complementary sequence in the 3' mRNA transcript. The strength of this bond depends on the sequence and number of seeds. For the most part, miRNA-mRNA interactions result in down-regulation though a small number cause up-regulation or complete destruction of the mRNA target.

The role of miRNA in cancer was first found in leukemia[69]. Since then, it has been discovered that altered expression of miRNA contributes to most, if not all, human cancers. Furthermore, it has been found that miRNA can either initiate carcinogenesis or drive disease progression[69].

Unlike somatic DNA mutations, miRNA expression is dynamic and both their expression and target may vary within the same cell depending on time or circumstance. This allows for significant signal amplification as a single protein may act via a small number of miRNAs to influence many genes[70].

Alterations in miRNA expression may themselves be driven by either genetic or epigenetic changes. Many miRNAs are located in genetically unstable sites where they are prone to deletion or rearrangement in cancer[71]. In addition, miRNA function may be affected by mRNA mutation in the target site. Epigenetically, many miRNA genes are located next to CpG islands where they may be prone to epigenetic silencing. This phenomenon has been documented to be relevant in urologic malignancy[72-75].

MiRNA genes may be located either within coding mRNAs or in the intergenic region. Approximately one-third are clustered while the remainder are solitary. In clusters, single events

may affect several miRNAs and subsequently thousands of protein targets.

Porkka et al. published the first report describing miRNA expression in prostate cancer in 2007[76]. They compared benign and malignant cells and found that many miRNAs were either up or down regulated. Hundreds of reports have subsequently looked at the role of miRNA in prostate cancer and at least 26 unique miRNAs have been implicated.

4.1 Apoptosis avoidance

One of the most important events in carcinogenesis is the avoidance of apoptosis. Thus far, at least 10 different miRNAs have been found to be involved in this process.

In many cases, this follows a cascade pattern. For example, up-regulation of the miR-17-92 cluster leads to over-expression of miR-20a which subsequently targets E2F1-3 transcription factors[77]. Then, depending on the cell cycle phase, reduced E2F1-3 results either in cellular proliferation or reduced apoptosis via p53 and caspase activity, thus creating an auto-regulatory feedback loop as E2F1-3 controls miR-20a expression. E2F1 expression is also down-regulated by miR-25 and miR-205[78,79].

Furthermore, miR-21 contributes to apoptosis through the p53 network in a mechanism that seems to be preserved throughout many malignancies[80]. In prostate cancer specifically, miR-21 has been found to target both PDCD4 (programmed cell death 4) and PTEN (phosphatase and tensin homologue) mRNAs in order to decrease apoptosis.

A recurrent theme in miRNA mediated genetic expression is multiple targeting and feedback loops. In apoptosis avoidance, this is seen in the miR-34 family whose expression is partly controlled by p53[81]. Loss of p53 activity results in decreased miR-34a expression which subsequently decreases targeting of the SIRT1 (silent information regulator 1) locus. As a result, up-regulated SIRT1 results in further down-regulation of p53 and decreased apoptosis. Due to this, miR-34a/b/c are down-regulated and induce their own effects.

4.2 Cellular pathways

Apart from apoptosis avoidance, cell cycle regulation, intracellular signalling, DNA repair and adhesion/migration are all affected by miRNA. *In vitro* experiments have shown that there is up-regulation of miR-221/222 in the PC3 cell line[82]. By targeting p27(kip1), these miRNAs induce cell proliferation through inhibition of this cell cycle checkpoint. Furthermore, miR-15a and miR-16-1 are down regulated in a majority of prostate tumors[83]. This results in an up-regulation of cyclin D1 which facilitates the G1/S transition and cellular proliferation. In addition, these miRNAs target WNT3a so their loss results in WNT activation which is carcinogenic. There is significant evidence that there is an interaction between miRNAs and key carcinogenic events – for example, miR-21 up-regulation can reduce apoptosis, induce proliferation and assist cell migration[84].

4.3 Androgen signalling

MiRNAs are intricately involved in a complex feedback loop involving androgen signalling. Androgen responsive miRNAs modulate the androgen pathway. For example, mi-125b contains an androgen-responsive element (ARE) within its promoter[85]. *In vitro* studies have shown that miR-125b up-regulation leads to androgen-independent growth in LNCaP cells and decreases apoptosis through targeting of BAK1, BBC3, and p53[86]. MiR-21 also contains an ARE in its promoter and, through multiple channels, may be involved in androgen insensitivity. MiR-141 was recently found to be the most strongly regulated by androgen signalling in cell culture and xenografts and is also over-expressed in prostate

cancer[87]. Interestingly, miR-141 is up-regulated in human prostate cancer. In addition, miR-146a acts upon ROCK1, a kinase involved in the development of castrate resistant prostate cancer.

Sun et al. found that there was up-regulation of miR-221/222 in androgen-resistant versus androgen-sensitive cells[88]. Manipulation of the levels of these miRNAs altered the cellular response to dihydrotestosterone (DHT), as measured by PSA and promoted the development of androgen-independence.

There is also crosstalk between miRNAs and other signalling pathways through shared transcription factors. ERBB-2 (Her2-neu) is a tyrosine kinase receptor that is over-expressed in some prostate cancers. Loss of miR-331-3p appears to up-regulate ERBB-2 expression. *In vitro* expression of miR-331-3p suppressed ERBB-2 expression and prevented androgen signalling[89]. This occurred in an androgen receptor (AR)-independent manner and was enhanced by the administration of bicalutamide. Looking at networks of related genes, Wang et al. found that miR-331-3p was among the central 20 RNAs altered between low- and high-risk prostate cancers[90].

5. SUMMARY

Clearly, to detail each of the genetic events or aberrations that may play a part in prostate cancer tumorigenesis is beyond the scope of this paper. Here we emphasised key derangements in both germline and tumor DNA as well as the role of epigenetic factors and miRNAs in prostate cancer development.

Moving forward, a better understand of the genetic events involved in prostate cancer will open opportunities for increasingly sophisticated biomarkers to both diagnose and risk stratify patients and for therapeutic targets and the development of novel treatments.

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Metabolomics and its application to the development of clinical laboratory tests for prostate cancer

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ABSTRACT

Introduction: There is a critical need to develop clinical laboratory assays that provide risk assessment for men at elevated risk for prostate cancer, and once diagnosed, could further identify those men with clinically significant disease.

Methods: Recent advancements in analytical instrumentation have enabled mass spectrometry-based metabolomics methodologies. Further advancements in chromatographic techniques have facilitated high throughput, quantitative assays for a broad spectrum of biochemicals.

Results: Screening metabolomics techniques have been applied to biospecimens from large cohorts of men comparing those individuals with prostate cancer to those with no evidence of malignancy. Work beginning in tissues has identified biochemical profiles that correlate with disease and disease severity, including tumor grade and stage. Some of these metabolic abnormalities, such as dramatic elevations in sarcosine, have been found to translate into biological fluids, especially blood and urine, which can be sampled in a minimally invasive manner.

Discussion: The differential abundances of these tumor-associated metabolites have been found to improve the performance of clinical prognostic/diagnostic tools.

Conclusion: The outlook is bright for metabolomic technology to address clinical diagnostic needs for prostate cancer patient management. Early validation of specific clinical tests provides a preview of further successes in this area. Metabolomics has shown its utility to complement and augment traditional clinical approaches as well as emerging genomic, transcriptomic and proteomic methodologies, and is expected to play a key role in the precision medicine-based management of the prostate cancer patient.

BACKGROUND ON THE HISTORY OF METABOLITES AS BIOMARKERS IN THE CLINICAL LAB

Specific metabolites have been recognized as clinically actionable biomarkers for over a century. While this field did not begin with the linkage of specific biochemicals to inborn errors of metabolism (many of which have profound pathologies, including developmental and neurological defects), Garrod showed that specific clinical presentations exhibited consistent biochemical fingerprints in blood or urine, such as the marked elevation of homogentisic acid in the urine of alkaptonuric subjects [1]. With the advent of tandem mass spectrometric methods, almost all newborns in the United States have blood samples analyzed for a panel of metabolic markers that are diagnostic for more than 30 of these rare but morbid diseases [2, 3].

Beyond newborn screening, standard clinical chemistry panels measure a number of biochemicals to assess human health. Urea and creatinine provide clinically useful information regarding renal function and glucose is frequently monitored as an indicator of diabetes. As described below, recent advances in analytical technologies have enabled the field of metabolomics which in turn has facilitated the discovery of biochemical markers of many diseases (including cardiometabolic disease and numerous malignancies). When developed into clinical assays, these metabolomic biomarkers are expected to play a role in precision medicine-based patient management [4-6].

CLINICAL NEEDS IN PROSTATE CANCER PATIENT MANAGEMENT

Case management uncertainties surrounding patients presenting with intermediate levels of PSA, create an urgent need for metabolite-based diagnostic tests which might identify prostate cancer and further discriminate between indolent and aggressive disease [7, 8]. The ability to measure metabolites which correlate with the grade and stage of prostate cancer, survival rates and frequency of recurrence, in a minimally-invasive biological specimen such as blood or urine would be of great value to optimize the allocation of healthcare resources to manage prostate cancer patients, both in preventing over-diagnosis and over-treatment and in selection of subjects for active surveillance.

Prostate cancer is the most common male malignancy with an estimated 240,000 new cases and more than 28,000 deaths in the United States in 2012 [9]. Clinical detection of prostate cancer increased following the widespread adoption of serum prostate-specific antigen (PSA) screening; however, a significant fraction of prostate cancers detected solely on the basis of an increased serum PSA are indolent. As a result of concerns of over-diagnosis and over-treatment, a new paradigm of active surveillance in patient management has emerged recently [10]. The resulting broad spectrum of treatment options (active

surveillance, focal therapy, radical surgery or radiation) has been developed in response to the increased detection of low-risk prostate cancer [11]; however, the current panel of diagnostic tests provides limited information regarding the progression potential (that is, the aggressiveness) of an individual's cancer. In a recent meta-analysis of eleven large clinical cohorts, Chou et al. concluded that PSA screening had no effect on prostate cancer-specific mortality [12]. Even when prostate cancer is detected, there is a significant clinical need to improve the accuracy of characterizing the biological potential of the tumor. Therefore, new tests are urgently needed to separate those individuals with aggressive prostate cancer from those with indolent disease.

PROSTATE-SPECIFIC METABOLISM IN HEALTH AND UPON MALIGNANT TRANSFORMATION

The healthy prostate is a hormone-sensitive exocrine gland that secretes a complex milieu of biochemicals into seminal fluid, including citrate, polyamines and *myo*-inositol [13]. The prostate's ability to accumulate intracellular citrate is unique amongst human tissues and is facilitated by zinc-mediated inhibition of aconitase, the citrate-metabolizing enzyme of the tricarboxylic acid cycle [14]. Early biochemical studies identified that citrate and other prostate-specific biochemicals are depleted from prostate adenocarcinoma [15, 16]. The loss of prostate-specific metabolic functions is one manifestation of dedifferentiation upon transformation of healthy prostate cells into prostate adenocarcinoma. Prostate cancer cells also accumulate a spectrum of biochemicals that are mechanistically associated with cellular growth and division pathways such as aerobic glycolysis (that is, the Warburg effect) [17] and membrane biosynthesis [18, 19]. Importantly, androgen receptor activity impacts prostate cancer through regulation of metabolism and biosynthesis [20].

Recent studies have identified relationships between many oncogenes and various metabolic pathways, which have led to the concept of metabolic reprogramming in cancer cells [21-23]. This reprogramming primarily reroutes intracellular metabolism to support cell growth and division however, evidence is emerging that tumors may also exploit metabolic pathways in neighboring cells [24, 25]. Together, cancer and stromal cells may operate in tandem; not only do the cancer cells multiply, but the extracellular matrix is remodeled and regional and systemic physiologic responses can be affected by metabolic products (for example, angiogenesis [26], and immunosurveillance [27]). The mechanistic linkages between individual biochemicals and specific pathophysiologic responses suggest that those metabolites (and possibly metabolic waste products, too) are released by clinically significant tumors.

METABOLOMICS IN PROSTATE CANCER

Recent advancements in analytical instrumentation have enabled the discipline of metabolomics, the high throughput, information-rich study of biochemical compounds and pathways. This suite of methodologies is not constrained to measuring metabolites from a single biochemical pathway, but can measure several hundred biochemicals from a single specimen. The application of these technology platforms to prostate cancer have been reviewed elsewhere [28-30].

Sreekumar et al. [31] conducted global metabolic profiling of benign prostate and prostate tumor to identify a more comprehensive catalog of metabolic alterations in prostate cancer and to determine how these metabolic changes relate to tumor development and progression. Their unbiased metabolite profiling study identified

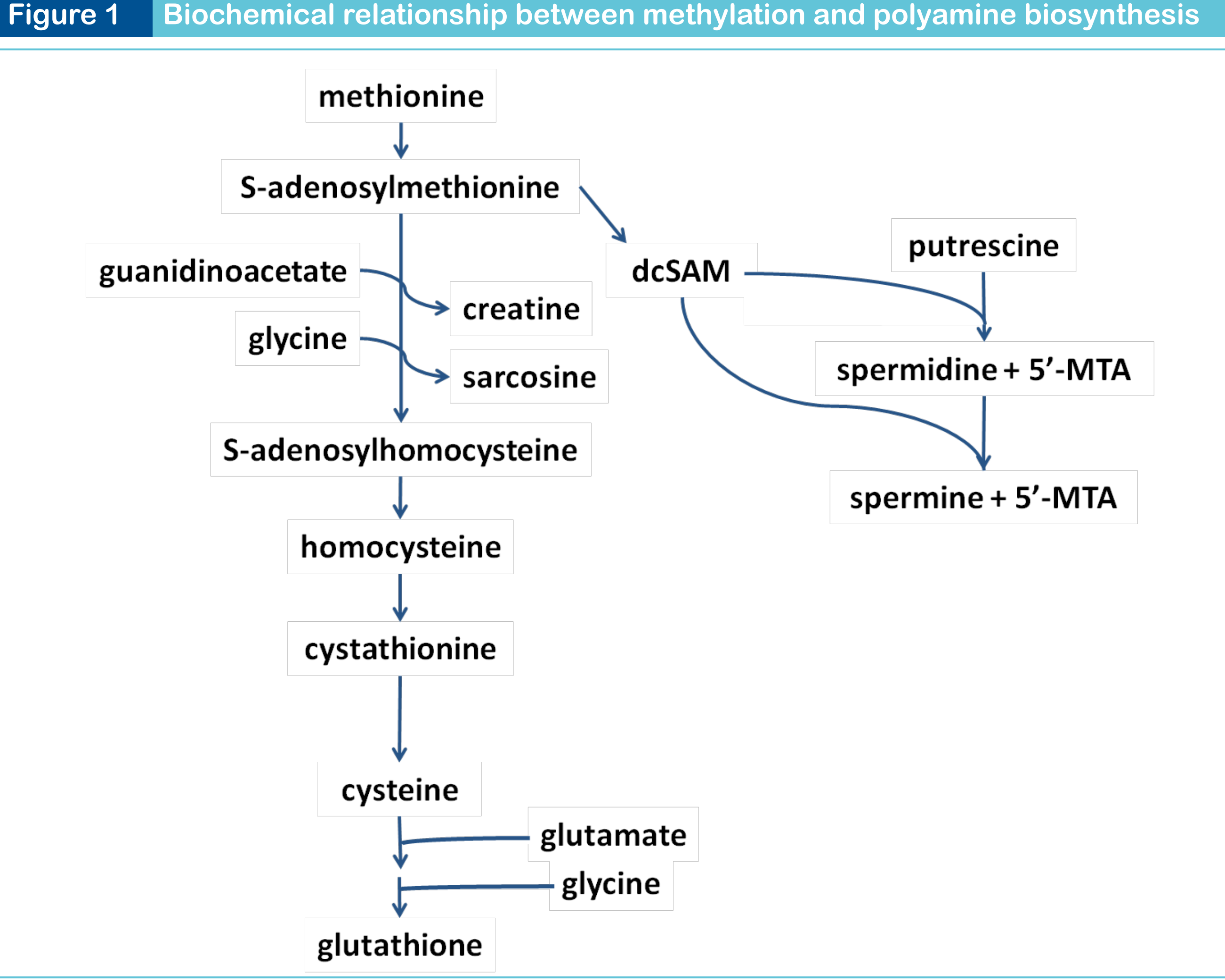
626 biochemicals; over 200 of these compounds exhibited statistically significant changes in pairwise comparisons of either benign tissue to localized prostate cancer or localized prostate cancer to metastatic sites. Six of these metabolites (sarcosine, uracil, kynurenine, glycerol-3-phosphate, leucine and proline) were found to increase across the spectrum of disease progression.

The increasing levels of sarcosine with disease progression were confirmed in an independent cohort of tissue samples using a quantitative gas chromatography-mass spectrometry (GC-MS) assay. Additional support for a role for sarcosine in prostate cancer aggressiveness has come from studies demonstrating the effect of glycine N-methyltransferase (GNMT) and sarcosine dehydrogenase (SARDH) levels on in vitro prostate cancer cell line sarcosine levels and invasive properties [31-33]. Sarcosine can be generated through methylation of glycine by GNMT. Conversely, sarcosine can be converted back to glycine through the action of SARDH. Overexpression of GNMT and knockdown of SARDH increased sarcosine levels and this was correlated with increased cellular invasiveness. An important role for GNMT and SARDH in prostate malignancies is suggested by findings that GNMT protein levels are elevated in tissue biopsies of prostate cancer and metastatic disease [33]. In addition, tumor expression levels of GNMT were found to correlate positively with PSA, stage, and Gleason score, while displaying a negative correlation to PSA-recurrence free survival [33]. The data generated in Sreekumar et al. provided evidence that global metabolomic profiling could identify new biomarker candidates of aggressive prostate cancer.

While the precise role of sarcosine in prostate cancer has yet to be delineated, it is likely related to methylation. Epigenetic silencing through methylation has been identified to play a significant role in the development and progression of many solid tumors including prostate [34, 35]. Glycine N-methyl transferase has recently been shown to harbor a functional androgen response element in its first exon allowing the expression of this gene to be driven by endogenous and synthetic androgens [36]. In histologically normal prostate and low grade prostate cancer, the GNMT protein is expressed in few cells and is primarily localized in the nucleus. Both the abundance and localization of GNMT is dramatically altered in a subset of high grade prostate tumors - GNMT was shown to be present in high abundance and present in the cytoplasm [33]. One interesting possibility is that the increase in sarcosine may be related to the reduction in polyamine abundance in prostate tumors. The metabolic intermediate S-adenosylmethionine (SAM) is used in both biochemical pathways: as a methyl donor in the GNMT reaction of glycine -> sarcosine and as an aminopropyl donor in the reaction of putrescine -> spermidine and spermidine -> spermine. Instead of consuming SAM for polyamine biosynthesis, the prostate cancer cell that can convert SAM to S-adenosylhomocysteine (by using SAM as a methyl donor) would also produce cysteine. This increase in intracellular cysteine abundance could facilitate increased glutathione biosynthesis and confer resistance to oxidative stress (Fig. 1).

A more recent study from our laboratory (McDunn et al. [37]) has extended the utility of global metabolite profiling to identify metabolic signatures of aggressive prostate cancer. Prostatectomy tissues from 331 tumors and 178 cancer-free tissues were subjected to global profiling to identify metabolite signatures associated with malignancy and tumor aggressiveness. Metabolic correlates to Gleason score, extracapsular extension, spread to seminal vesicles and/or lymph nodes and 5-year progression-free survival were identified. For each of the tumor aggressiveness characteristics, a set of metabolites was identified that differentiated between comparator groups. For instance,

Figure 1 Biochemical relationship between methylation and polyamine biosynthesis



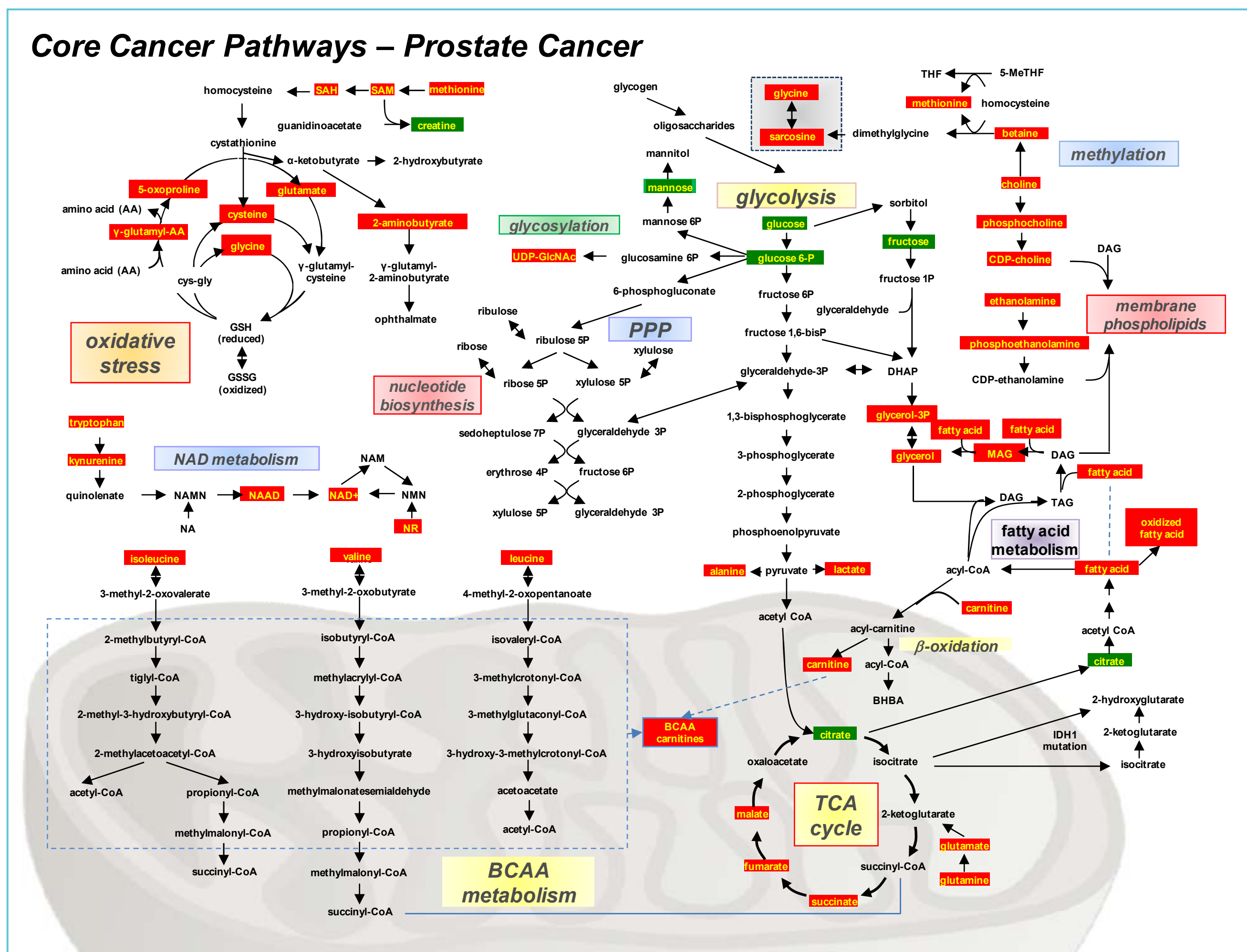
S-adenosylmethionine (SAM) is a key intermediate in both pathways. Loss of polyamine production may result in increased utilization of SAM for methylation, which in turn could result in increased intracellular cysteine and glutathione (dc-SAM, decarboxylated S-adenosylmethionine; 5'-MTA, 5'-methylthioadenosine).

in Gleason score comparisons, 28 metabolites (25 were increased in abundance; 3 were decreased in abundance) were found to correlate to Gleason pattern progression from 6 → (3+4)7 → 7(4+3) → 8, at statistically significant levels. Among the metabolite classes correlating best with Gleason pattern progression were amino acids and their catabolites, energetics related compounds, lipid components and metabolic stress associated compounds (Fig. 2)

All metabolites selected, using clinical criteria that separate clinically significant prostate cancer from indolent disease, were subjected to hierarchical clustering analysis. This approach partitioned the clinical subjects into three groups: one group was enriched for subjects with less aggressive disease and two groups were enriched for subjects with more aggressive disease, each with a specific pattern of dysregulated metabolites (Fig. 3).

Exploratory analysis of the metabolite profiling data was also carried out to determine whether metabolites could augment the performance of clinically useful prediction tools [38, 39]. A 4-metabolite panel was found to augment the performance of the commonly used Partin probability

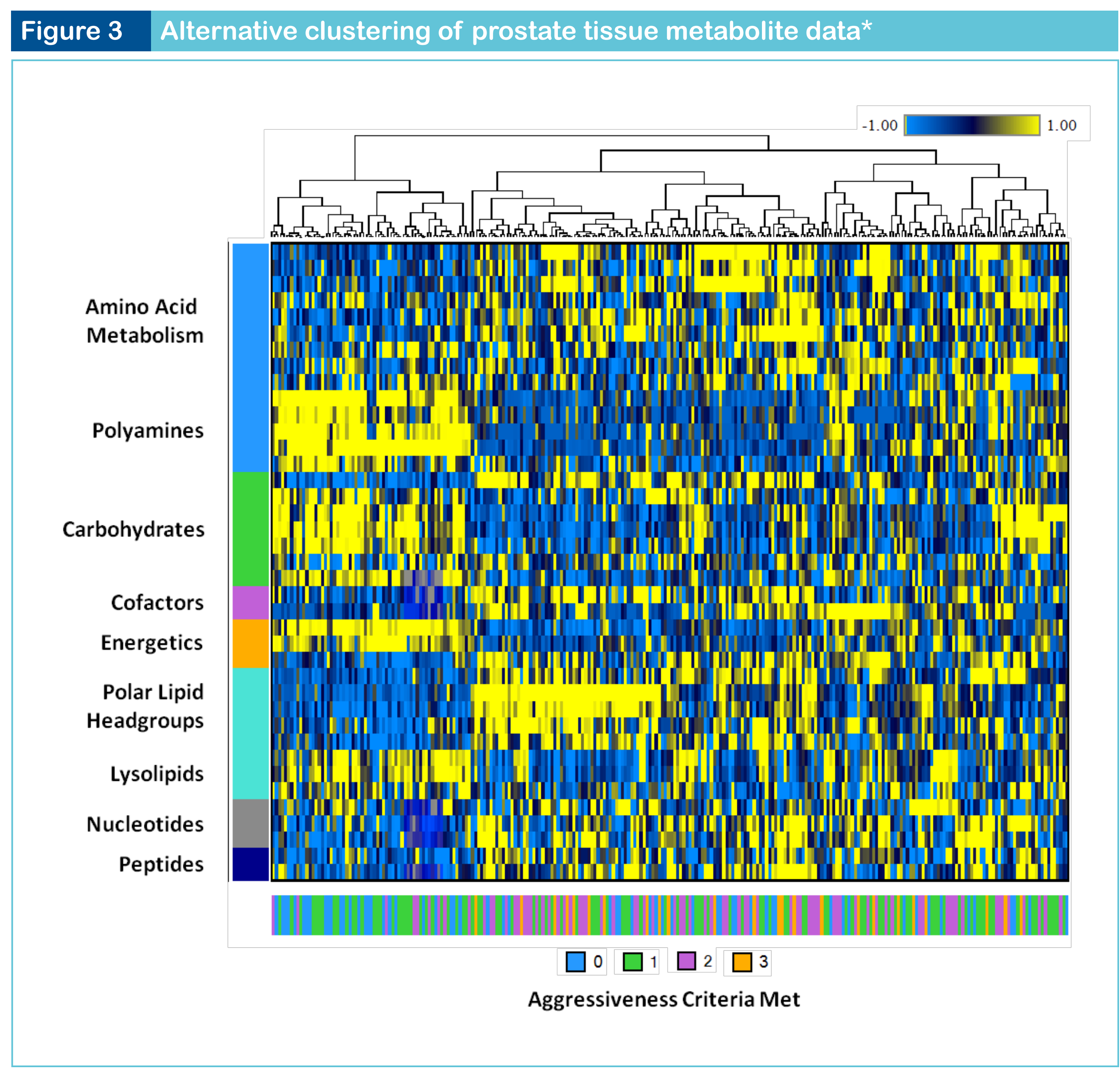
Figure 2 **Changes in core metabolic pathways in prostate cancer relative to normal prostate tissue**



Core metabolic pathways that are often altered in the malignant tissues are displayed. Metabolites in red boxes have been observed to be increased in prostate tumors relative to cancer-free prostate tissue, while green boxes indicate a decrease (taken from Ref. 28). Clusters of elevated metabolites are seen in pathways related to membrane phospholipid synthesis, methylation and oxidative stress. Increases in branched chain amino acid (BCAA) metabolism are suggested by an increase in BCAA related carnitines and an increase in the three BCAAs. TCA cycle intermediates were elevated along with glutamine and glutamate which can feed the TCA cycle through 2-ketoglutarate. Citrate, which acts as an intermediate in the TCA cycle and is also utilized in fatty acid synthesis, was observed to be lower in prostate cancers. Unlike many tumor types, prostate cancer tissue did not display a large increase in glycolysis intermediates typical of a shift in energy metabolism away from mitochondrial oxidative phosphorylation and toward aerobic glycolysis – although lactate and alanine which can be markers of increased aerobic glycolysis were elevated. The inter-conversion of glycine and sarcosine is highlighted in the dashed box.

nomogram for organ-confined disease, increasing the area under the receiver operator characteristic curve (AUROC) from 0.53 (clinical data alone) to 0.62 (clinical data plus metabolites). At a sensitivity of 90%, the Partin table had a specificity of 11% while the metabolites had a specificity of 17%. The Partin table and metabolites had similar performance at a specificity of 90%, (15% and 17% sensitivity, respectively). A separate 3-metabolite panel was found to enhance the AUROC of a Han 5-year progression-free survival nomogram from 0.53 (clinical data alone) to 0.64 (clinical data plus

Figure 3 Alternative clustering of prostate tissue metabolite data*



from Ref. [28], showing associations between metabolite classification (Y-axis) and tumor aggressiveness (an aggregate variable containing 1 point for each of the following criteria met: Gleason grade > 3+4; pT2; pT3. In general, less aggressive prostate tumors have higher levels of polyamines, simple sugars and lysolipids while more aggressive prostate tumors are characterized by elevated amino acids, polar lipid head groups, and cofactors.

metabolites). Interestingly, the Han table and the metabolites had similar performance at 90% sensitivity, but the metabolites outperformed the Han table at 90% specificity (with sensitivities of 23% and 11%, respectively).

These results will clearly require validation in additional studies in independent cohorts. However, the findings from McDunn et al. suggest that panels of metabolites may enhance prediction of clinical endpoints and more effectively stratify prostate cancer subtypes, in terms of their aggressiveness and biological potential. Furthermore, these data suggest that there may be more than one metabolic phenotype of clinically significant prostate

cancer, as defined by unique metabolomic signatures. This finding may help map treatment strategies for individual patients, and suggests that metabolomics may play a vital role in the application of precision medicine for prostate cancer patient management.

TRANSLATION OF STUDIES IN TUMOR TISSUES TO CLINICAL LABORATORY TESTS

Defining prostate cancer metabolic changes has most frequently focused on the interrogation and characterization of biochemical changes in tissue samples, but ideally, metabolite markers of prostate cancer could be detected and monitored in more easily accessible specimens, such as blood or urine. Sreekumar et al. [31] found that sarcosine levels were elevated in urine and urine sediments in patients with prostate cancer, relative to specimens from patients with no evidence of malignancy. The increase of sarcosine levels in the urine sediments of men with prostate cancer was recently confirmed in an independent cohort of patients [32]. Additional studies have shown that urine and urine sediment sarcosine are correlated to biopsy findings of prostate cancer and can improve the predictive accuracy of other diagnostic modalities, including the measurements of PCA3 and percent-free PSA [40-42].

It is important to note that there is some inconsistency in the findings of the significance of sarcosine and its association with prostate cancer development and progression, as at least one clinical study has been reported where urine sediment sarcosine was not associated with the biopsy-based diagnosis of prostate cancer [43]. However, these discrepancies may be related to methodological differences in study design (patient populations), specimen preparation and analytical techniques. As Issaq et al. have pointed out, none of these manuscripts have published their methods in sufficient detail for critical comparisons to be made [44].

In addition to the work on sarcosine, a few studies have suggested that blood-borne metabolites may have utility as biomarkers to monitor the development and progression of prostate cancer [45-47].

ANALYTICAL DEVELOPMENT OF QUANTITATIVE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) ASSAYS FOR SARCOSINE AND OTHER METABOLITES FROM URINE SEDIMENT

The clinical studies that led to the demonstration that sarcosine is a urine marker of prostate cancer demonstrated that sarcosine in urine sediments has equivalent or better performance than intact urine or urine supernatants in separating patients with prostate cancer from patients with benign pathologies. This finding led to the development of a metabolite-based prostate cancer risk stratification tool using urine sediments.

Sediment pellets from urine specimens can be limiting and ultra-sensitive techniques are required for the analysis of metabolites from these specimens. Previously, GC-MS had been used for the metabolomic analysis of urine sediment pellets, due to the enhancement in sensitivity that derivatization can provide for small molecules. However, with the concept of an optimal clinical laboratory test in mind, the techniques required for the preparation of derivatized GC-MS/MS sample extracts and the lengthy run times necessary for GC analysis do not lend themselves to the development of a high-throughput diagnostic test.

In addition to the advances in chromatographic and mass spectrometric technology that enabled the development of a screening metabolomics platform, other advances in analytical

instrumentation have facilitated the use of LC-MS/MS in ultrasensitive, quantitative metabolite measurements. Ultra-high performance LC-MS/MS methods have been developed to measure the metabolites sarcosine, alanine, glutamate, and glycine in their underivatized form, and have demonstrated greater analytical sensitivity and accuracy that is comparable to the traditionally used derivatized GC-MS method, with an analyte correlation of >0.99. (Ford et al, manuscript in preparation). Compared to published GC-MS methods, these changes can increase the throughput of the assay by approximately 10-fold and can significantly decrease sample preparation time and consumables costs, making the development of cost-effective clinical laboratory tests more feasible. The metabolites sarcosine, alanine, glutamate, and glycine can be measured in concentrations as low as 100 picograms per sample, and the 500-fold analytical range of all of the biochemicals is suitable for measurement in typical urine sediment samples. The increase in analytical sensitivity also enables the application of this technique to routine clinical laboratory testing: in contrast to the benchmark GC-MS assay, these changes and improvements in measurement sensitivity decreased the frequency of patient samples reported as “below the detection limit” from 25% to less than 1%.

LABORATORY VALIDATION OF A METABOLOMIC PROSTATE CANCER CLINICAL TEST

The method to quantitatively measure the 4-metabolite panel associated with the presence of prostate cancer was validated and implemented in the Metabolon clinical laboratory as the Prostarix™ clinical test for the assessment of the likelihood that a patient will have a positive finding for a malignancy after a prostatic biopsy. The likelihood score is derived from a logistic regression algorithm of the LC-MS/MS-based metabolomic measurements. Urine sediment quality control samples were prepared from bulk pools of urine to obtain sediments that represent the low, medium, and high levels of analyte measurement. The performance characteristics of the analytical protocol were established by Clinical Laboratory Standards Institute (CLSI) methods, over 23 days, with % CVs in the quality control urine sediments typically ranging from 4-16% and overall Prostarix score % CV less than 6%. The performance of the analytes and the overall Prostarix score are continuously monitored using quality control samples in the clinical laboratory as well as clinical samples from an independent test cohort.

The clinical performance of this assay was determined in a patient cohort with PSA levels between 2 and 15 ng/mL who were being considered for a prostate biopsy. Analyte abundances were associated with biopsy outcomes and these measurements were combined to develop a logistic regression algorithm to generate a Prostarix Risk Score. The test performance and algorithm development were validated in an independent cohort. Results demonstrated that individuals with Prostarix scores >60 were 3.5 times more likely to have prostate cancer detected on biopsy compared to those individuals with Prostarix scores <40. (McDunn et al, manuscript in preparation). The performance of the metabolite panel (AUROC = 0.64) was superior to either PSA alone (AUROC = 0.53) or the clinical parameters that are used to calculate individualized risk of prostate cancer based on the PCPT trial (AUROC = 0.61) [39]. When available, such as in the case of a patient undergoing a repeat biopsy after a previous negative biopsy, the TRUS-measured prostate volume and the patient’s most recent PSA measurement can be used along with the metabolite measurements to generate a Prostarix Plus score, which can further improve the stratification into higher risk and lower risk patient groups

(AUROC = 0.78). In the test population, both Prostarix and Prostarix Plus had performance significantly greater than either PSA or the PCPT risk calculator. At 90% sensitivity, the specificity of Prostarix was 28% while Prostarix Plus had a specificity of 41%, and at 90% specificity, the sensitivity of Prostarix and Prostarix Plus were 24% and 47%, respectively.

CONCLUSION AND FUTURE DIRECTIONS

Metabolomics, the measurement of small biochemical compounds in diverse biospecimens, may allow us to better understand the behavior of metabolites, the biochemical byproducts of cellular metabolism. The determination of these molecules is important because it may provide a fingerprint or signature to inform researchers and clinicians of the biochemical cellular activities on-going in healthy and diseased tissues. Both global profiling (the measurement of thousands of small molecules) and targeted analyses (directed, quantitative measurements of a limited panel of biomarkers) may provide a critical opportunity for improving our ability to detect, characterize and potentially manage and treat prostate cancer. Differences in the prostate cancer subtype metabolic behavior may be correlated with characteristic metabolomic biochemical signatures and these signatures could aid in the characterization and classification of tumors. The measurement of small biochemicals reflects a phenotype that should be associated with the development and progression of prostate cancer. These biochemical changes may indicate, and serve to integrate, alterations in the genome, transcriptome and proteome that lead to downstream metabolic dysregulation. Our ability to identify and accurately quantitate the relevant biomarkers will depend on the application of a wide spectrum of analytical and bioinformatic techniques to fully interrogate the entire metabolome. In addition, the ability to reproduce and confirm results from individual laboratories will only be possible with comprehensive published descriptions of methodologies and informatic approaches.

Since the metabolic spectrum should be highly responsive to changes in physiologic status, the development and progression of a prostatic malignancy, conversion to a higher aggressiveness phenotype, and response to treatment, is anticipated to be assessable by baseline measurements and serial monitoring of metabolic biomarkers over the course of the management of the prostate cancer patient. However, there are clearly strengths and limitations to the ultimate objective of developing metabolomic-based clinical diagnostics for prostate cancer. Among the conditions that favor efforts to develop such tests are the high levels of analytical sensitivity and specificity of measurements that are available with the recent advances in instrumentation and bioinformatics, the ability to measure these biomarkers in a variety of biospecimen types (blood, urine, tissue, etc.), and the rapidly expanding application of these techniques into the clinical laboratory.

The challenges to the implementation of this technology remain profound: significant work must be done to establish and characterize potential confounding factors to the accurate measurement of the biomarkers of prostate cancer metabolism, including the influence of diet, medications, supplements, physical activity, sampling times, diurnal variation, specimen preparation, stability, etc. The effects of numerous pre-analytical variables must be assessed and minimized, in the effort to develop robust, reproducible and clinical useful diagnostic methods. In addition, issues of standardization, throughput, and instrument costs will potentially serve as near-term impediments to the broader introduction of the technology into the clinical laboratory.

Since the initial publication identifying sarcosine as a mechanistic biomarker of prostate cancer progression, several researchers have developed analytical methods to measure sarcosine. Early attempts to characterize the clinical utility of sarcosine in different matrices and different clinical populations had mixed results, and the possible reasons for this have been laid out elsewhere [28, 44].

A limited number of clinical studies evaluating the relationship between serum sarcosine and prostate cancer in men have given disparate results (for example, [48, 49]) and more work will be required before the clinical utility of sarcosine in this matrix is fully appreciated.

As described above, there has been considerable effort invested into both the pre-analytical and analytical methods for sarcosine quantitation during the development of Prostarix.

The pre-analytical method was found to be sensitive to contamination and operator-to-operator variability whereas the analytical method required extensive development to achieve an acceptable lower limit of quantitation (methodologic optimization significantly reduced instrument background and minimized matrix suppression and chemical interferences).

Despite these challenges, a number of groups have shown that sarcosine in post-DRE urine specimens can be used to stratify prostate cancer risk (Table 1).

All considerations accounted for, the outlook for the use of metabolomic technology in addressing the clinical diagnostic needs for prostate cancer patient management remains bright. Specific and characteristic metabolic changes have been described that are associated with the prostate and its malignant transformation. Early validation of specific clinical tests provides a preview of further successes in this area. The ability to complement and add important clinical insight to other traditional clinical approaches and emerging techniques applying genomic, transcriptomic and proteomic methodologies makes a strong argument for the potential for metabolomics to improve the practice of precision medicine for prostate cancer patients.

Table 1 Clinical studies of post-DRE urine sarcosine and its ability to stratify men with regard to subsequent biopsy outcomes

Number of subjects, N (Biopsy-positive / Biopsy-negative)	**Analytical approach**	**AUC**	**Reference**
131 (86, 45)	LC-MS/MS (derivatized)	0.67	[41]
110 (71, 39)	LC-MS/MS (derivatized)	0.70	[40]
139 (106, 33)	GC-MS (derivatized)	0.63	[43]
93 (49, 44)	GC-MS (derivatized)	0.71	[31]
56 (33, 23)	GC-MS (derivatized)	0.82	[42]
345 (211, 134)	GC-MS (derivatized)	0.71	[32]

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Testosterone deficiency – establishing a biochemical diagnosis

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ABSTRACT

Testosterone deficiency is a common and often unrecognized disorder impacting the lives of many men. Symptoms related to low testosterone are relatively non-specific and clinicians must therefore ensure that a patients' symptomatology is supported by a biochemical profile suggestive of testosterone deficiency. There are many options available to determine a patient's testosterone level and laboratories will vary in the type of biochemical assessment they provide. In assessing patients with suspected low testosterone, the presence of symptoms and a low total testosterone is usually sufficient to initiate therapy. In equivocal cases, measurement of free or bioavailable testosterone with a reliable assay can further clarify the clinical picture. By understanding the differences between total, free and bioavailable testosterone, and the accuracy and reliability of their measurement, clinicians can better interpret their patients' biochemical testosterone profile.

INTRODUCTION

Testosterone Deficiency (TD), also known as Androgen Deficiency in the Aging Male (ADAM), hypogonadism and andropause is estimated to affect 10% of men older than 30 years of age and up to 40% of men older than 70 years of age[1]. Despite its prevalence, it is estimated that only 5-10% of men with low testosterone are being treated[2]. It is now well established that TD negatively impacts sex drive, erectile function, energy levels, mood, cognition, muscle mass, bone density and fat accumulation[3]. Further, it has been demonstrated that correcting testosterone to normal physiologic levels can lead to improvement of many of these conditions. Despite such a prevalent, reversible problem, most men are not evaluated or treated. Moreover, even when the diagnosis is suspected, primary care physicians, endocrinologists and urologists face the challenge of making an accurate biochemical diagnosis due to controversies regarding testosterone measurement. In this review paper, we aim to summarize the different options with regards to testosterone measurement and their associated strengths and limitations. Informed clinicians should be able to request and interpret biochemical results within this context to best assess and treat patients with TD.

WHAT IS TESTOSTERONE?

Understanding the concepts and controversies surrounding the biochemical evaluation of testosterone deficiency requires a fundamental understanding of the physiology of testosterone production, homeostasis and action. Testosterone is critical in the male for its contribution to libido, mass muscle, fat distribution, mood, energy and sexual function. Testosterone is largely (90%) produced by Leydig cells within the testes. Gonadotropin-releasing hormone (GnRH), released by the arcuate nucleus of the hypothalamus regulates the pituitary production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates the production of testosterone while FSH binds to Sertoli cells and promotes spermatogenesis. Approximately 10% of male androgens are produced by the adrenal glands.

Testosterone is metabolized to dihydrotestosterone (DHT) and estradiol (E2) - active metabolites that provide negative feedback at the level of the pituitary. Testosterone exerts its effects through binding androgen receptors in target cells. In some instances testosterone is a prohormone and is converted by 5-alpha reductase enzymes to DHT. Two distinct 5-alpha reductase enzymes exist. Type 1 is androgen independent and is located in the skin, liver and brain. Type 2 is controlled by androgens and is distributed in the prostate, seminal vesicles and testicles. DHT has a 10-fold greater affinity for androgen receptors than testosterone.

Testosterone exists in the bloodstream in two forms, bound and unbound. The vast majority of testosterone is bound to plasma proteins with approximately 2-3% of total testosterone being unbound. The unbound component is referred to as free testosterone (FT) and is thought to be the component that has access to cells and possesses androgenic action. Testosterone that is complexed to albumin is weakly bound and has androgenic potential. Testosterone bound to sex hormone binding globulin (SHBG) is tightly bound and is essentially inactive. Bioavailable testosterone (BT) refers to both free testosterone and that which is bound to albumin.

TESTOSTERONE MEASUREMENT

Measuring serum testosterone has limitations both in theory and in practice. Circadian rhythms influence testosterone with levels peaking in the morning[4]. Reflecting a conservative approach to diagnosis, testosterone blood measurement is usually requested in the morning

hours between 8-10 am. However, more recently, there is evidence that as men age, such diurnal variations are blunted to some degree[5]. Interindividual variability in testosterone measurements has been demonstrated within the same week[6]. Acute illness will depress testosterone levels and misrepresent one's true hormonal status[7]

Many expert advisory panels and clinical guidelines have avoided strictly defined cut-off values for "normal" testosterone due to difficulties in measurement techniques and variability between institutions and patients over time. What form of testosterone should be measured and what method of measurement should be employed remain controversial but vitally important issues in the diagnosis and treatment of testosterone deficiency syndrome.

VARIATION OF TESTOSTERONE LEVELS BASED ON PATIENT FACTORS

Testosterone levels have been shown to vary with many pathological and physiological processes. From age 40, mean serum total testosterone levels have been shown to gradually decline at a rate of about 1% per year. Moreover, with age, SHBG increases thus lowering both bioavailable and free testosterone by removing more testosterone from the bioavailable pool, reducing the total amount capable of exerting androgenic activity. Various factors can alter physiological levels of SHBG and, with them, levels of bioavailable testosterone (see table 1). SHBG is found to be decreased in obesity, acromegaly and hypothyroidism. Aging, liver disease, hyperthyroidism, and anticonvulsant use all increase SHBG level and therefore decrease the bioavailable levels of testosterone[8].

Lifestyle choices and comorbid conditions may also lead to lowered levels of testosterone. Tobacco, alcohol use, caffeine, obesity and stress have all been demonstrated to be associated with lower testosterone levels[9]. Type 2 diabetes, hypertension and sleep apnea are independent risk factors for declining testosterone levels[10]. The Hypogonadism In Men (HIM) study found the odds ratio for having low total testosterone to be 1.84 for hypertension, 2.09 for diabetes, and 2.38 for obesity[11]. A significant association between Vitamin D and hypogonadism has been described in well-designed studies[12].

TESTOSTERONE DEFICIENCY - SYMPTOM INVENTORIES

A number of diagnostic inventories or questionnaires exist for recognizing symptoms of testosterone deficiency. As a group, these inventories have been shown to have high sensitivity

Table 1 Conditions impacting sex hormone binding globulin

Conditions that increase SHBG	Conditions that decrease SHBG
Aging	Obesity
Liver Disease	Acromegaly
Anorexia	Anabolic Steroids
Anticonvulsant use	Diabetes
Hyperthyroidism	Hypothyroidism

in diagnosing a testosterone deficient state, but lack specificity for the condition[13]. The three most commonly used inventories currently are the Androgen Deficiency in Aging Males (ADAM)[14], the Aging Male Scales (AMS)[15], and the questionnaire of the Massachusetts Male Aging Study (MMAS)[16]. These inventories address the main symptomatology associated with testosterone deficiency (see table 2).

OPTIONS IN MEASURING TESTOSTERONE

Total testosterone

Total testosterone measures all forms of testosterone in the serum, both bound and unbound. Due to cost and availability, clinical evaluation of a patient suspected to be testosterone deficient generally begins with total testosterone measurement. Assays for total testosterone in plasma pose a number of challenges. Concentration of total testosterone can vary throughout the day. Steroids of similar structure to testosterone have been shown to cause assay interference with some assays. Further, validated age and/or gender corrected normal ranges do not currently exist using a standardized assay and there is no universally recognized testosterone calibrating standard thus complicating the use of thresholds for normal values. Lastly, conditions that affect levels of SHBG may ultimately skew the interpretation and significance of the total testosterone measurements. For example, as SHBG increases with age, total testosterone may be determined to be "normal", yet bioavailable levels of testosterone may be low.

Total testosterone can be measured through immunoassays or mass spectrometry. Immunoassays can either be performed directly by radioimmunoassay (RIA), enzyme-linked immunosorbent

Table 2 ADAM questionnaire

Androgen Deficiency in Aging Males (ADAM)	Patient Response
Do you have a decrease in libido?	Yes or No
Do you have lack of energy?	Yes or No
Do you have a decrease in strength or endurance?	Yes or No
Have you lost height?	Yes or No
Have you noticed a decrease in your enjoyment of life?	Yes or No
Are you sad and/or grumpy	Yes or No
Have you noticed a deterioration in your ability to play sports	Yes or No
Are you falling asleep after dinner?	Yes or No
Has there been a deterioration in your work performance?	Yes or No
Are your erections less strong?	Yes or No

assay (ELISA) or chemiluminescence immunoassay (CLIA) or after extraction and chromatography. The extraction and/or chromatography procedures allow for the removal of interfering proteins and the use of a large serum samples to increase measurement sensitivity. It is widely agreed that adding extraction and chromatography steps increases the accuracy of the direct assay[17]. Using extraction/chromatography or mass spectrometry, however, requires technical expertise, special facilities with waste disposal capabilities, and adds significantly more cost. Direct assays are technically simple, automated and inexpensive compared to those combined with extraction and/or chromatography. They are also significantly quicker, catering to busy clinics and laboratories. Direct assays, however, may overestimate actual testosterone levels and show limited accuracy at lower testosterone levels[18].

While there are no universally recognized lower and upper limits to total testosterone values, it is generally agreed that serum total testosterone values greater than 15 mmol/L are unlikely to be associated with significant clinical testosterone deficiency. A symptomatic male with total testosterone between 8 mmol/L and 15 mmol/L can reasonably be offered a trial of therapy. Measuring SHBG, BT or FT may be of benefit in symptomatic individuals with normal total testosterone and conditions that may affect SHBG levels.

Free testosterone

Free testosterone (FT) refers to the testosterone that is unbound. In most men, this represents 2-3% of total testosterone. The general consensus is that FT best correlates to the true androgenic state of the patient. There are three ways of measuring FT- indirectly by equilibrium dialysis, directly using an analog-based RIA (analog FT), or through standardized calculations. Equilibrium dialysis is costly and manual but remains the gold standard in measuring FT. Equilibrium dialysis involves adding radiolabeled testosterone (3 H-T) to the sample being assayed. The free and bound 3H-T is then separated after equilibrium is attained. The percentage of free 3 H-T is multiplied by the total testosterone to calculate the FT of the sample. This methodology is not used clinically due to cost and convenience. It is currently limited to mainly reference laboratories but serves as the standard in all investigational studies of testosterone measurement.

The direct analog-based RIA method has been in widespread use in Canada for over a decade[19]. This assay consists of adding a radiolabeled testosterone analog to an unextracted serum sample. The direct analog-based assay has received criticism from experts due to the fact that its results are consistently lower than those obtained by equilibrium dialysis[20]. However, some experts feel that as long as analog FT results are interpreted with that correction in mind, they are clinically valuable. Studies comparing the analog FT immunoassay showed a strong and clinically meaningful correlation to the equilibrium dialysis when compared to the calculated FT[21]. Due to numerical differences each test would require unique reference values. A recent investigation has demonstrated high correlation between calculated FT and direct analog-based RIA measured FT when compared to equilibrium dialysis, the gold standard in FT measurement[22].

Due to the cost and effort associated with empiric measurement of free testosterone, calculated free testosterone is a reasonable and far more commonly performed method. By measuring total testosterone, SHBG and either assuming an albumin value or measuring it, a free testosterone calculation can be performed. Studies have shown that application of standardized albumin levels leads to very little variation in calculated free testosterone and is clinically acceptable[23]. There are many online testosterone calculators

available for clinical use[24]. Laboratories are now independently reporting calculated free testosterone which obviates the need for clinicians to look up and implement tedious equations. The Vermeulen and Sodergard algorithms are the most commonly used in both clinical practice and research protocols[25].

In summary, direct assays for free testosterone are simple, rapid, and can be automated. Equilibrium dialysis is relatively expensive and technically demanding but remains the gold standard for free testosterone measurement. Calculated measurements, while useful, are limited by the accuracy of measurement of total testosterone and SHBG but have also demonstrated good correlation to equilibrium dialysis (see table 3).

Bioavailable testosterone

Bioavailable testosterone (BT), which consists of FT plus albumin-bound testosterone, can be measured directly by adding 3H-T to the serum sample and precipitating out the SHBG-bound testosterone with ammonium sulfate. The fraction of 3H-T not precipitated out is used to calculated bioavailable testosterone by multiplying it by the total testosterone value obtained in a separate sample. This assay is costly and requires significant effort. As a result, even though the direct measurement of bioavailable testosterone is currently the predominant method for measuring bioavailable testosterone, most clinicians and laboratories rely on FT or calculated FT measurement in place of bioavailable testosterone.

TREATMENT

The primary goal in treating patients with TD is symptom improvement by achieving physiological levels of testosterone. Currently in Canada, injectable, transdermal and oral formulations are options for testosterone replacement (see table 4).

Intramuscular injections (testosterone cypionate and testosterone enanthate) are cost effective and long acting. Due to the pharmacokinetics of injectables, serum testosterone may be supra-physiological immediately at the beginning of the injection cycle and may be subtherapeutic towards the end of the cycle, leading to recurrence of TD symptoms. Some patients find

Table 3 Options in measuring free testosterone

Method	Description	Strength	Weakness
Direct Assay/ RIA (Analog T)	Radiolabeled testosterone analog is added to unextracted sample	Cheap, widely available, quick	Results consistently lower than equilibrium dialysis results
Equilibrium Dialysis	3H-T added to sample, free and bound separated and percentage calculated	Gold standard	Expensive, labour intensive, dependent on the TT assay,
Calculated Free T	Automated established equations using TT, albumin and SHBG	Correspond well with equilibrium dialysis, less expensive	Depended on accuracy of input variables, multiple equations available and not standardized

Table 4 Treatment options

Treatment	Advantages	Disadvantages
Injectables (Testosterone cypionate, Testosterone enanthate)	• Inexpensive • Effective • Long acting (24 weeks)	• Can produce supraphysiologic levels at cycle beginning • Waning effect at cycle end • Require injection
Oral (Testosterone Undecanoate)	• No injection required	• Absorption issues • May induce high levels of DHT • Twice daily dosing
Transdermal (Androderm (patch), Androgel, Testim, Axiron)	• Easy to apply • Consistent testosterone levels	• Minor skin reactions

the idea of weekly or monthly injections daunting and there is a higher risk of erythrocytosis.

In Canada, oral testosterone undecanoate is approved but may lead to supraphysiologic levels of DHT[26]. By design, it bypasses the liver through lymphatic absorption to enable delivery to the systemic circulation. The oral formulation should be taken with a high fat meal (at least 20 mg of fat) to promote absorption and clinical response. Due to a short half-life, the oral formulation requires multiple doses per day.

Transdermal products have demonstrated both effective therapeutic control of testosterone levels and high patient satisfaction. AndroGel and Testim are both gel-based products approved in Canada and provide steady-state, physiologic levels of testosterone. They are very well tolerated with minor skin reaction in a minority of patients. Application is recommended on the shoulder, upper arms or abdomen. The transdermal patch (Androderm) has a similar profile to the gel-based products but has been shown to cause significantly more local skin reaction. Lastly, Axiron is a transdermal preparation made specifically for application to the underarms. By applying to the underarms there is theoretically less risk of secondary exposure by skin-to-skin contact with another individual.

MONITORING

A diagnosis of male breast cancer or prostate cancer represents absolute contraindications to testosterone replacement therapy. Prior to initiating testosterone therapy, physicians are recommended to measure patient's prostate-specific antigen (PSA) and perform a digital rectal exam (DRE) (see table 5). Testosterone therapy may cause erythrocytosis[27] and patients should have their complete blood count monitored during therapy. Within the first year of therapy monitoring should occur about every 3-6 months and include evaluation of serum testosterone, hemoglobin and hematocrit, PSA and prostate health (by DRE). Liver function tests are not required with modern testosterone formulations. Testosterone may worsen sleep apnea and congestive heart failure and patients should have those conditions addressed prior to initiating therapy. Further, exogenous testosterone is not recommended for men seeking fertility as sperm production may be reduced.

Table 5 Testosterone monitoring

Testosterone monitoring	
Baseline	Symptoms, Testosterone, DRE, PSA
Follow-up (initially q3 months and then q6-12 months)	Symptom response, CBC, DRE, PSA

CONCLUSION

Testosterone deficiency is common yet most men are not offered evaluation or treatment. The symptoms associated with TD are non-specific and can be easily overlooked or misattributed to other medical or psychosocial causes. Aside from symptomatic relief, treating low testosterone can offer other significant health benefits.

There are many options to determine the biochemical level of testosterone. Measurement of total testosterone represents a reasonable initial screening assay for most men with convincing symptoms of testosterone deficiency. In cases where the total testosterone is equivocal despite consistent symptomatology, assessment of free or bioavailable testosterone may be informative. Clinicians need to be familiar with the various options to measure a patient's testosterone status and an appreciation for the strengths and limitations of the assays used to determine such measurements.

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Clinical relevance of trace bands on serum electrophoresis in patients without a history of gammopathy

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Non-standard abbreviations:
IFE – immunofixation
MGUS – monoclonal gammopathy of undetermined significance
SMM – smoldering multiple myeloma
SPE – serum protein electrophoresis
TFS – trace, faint, suspicious

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ABSTRACT

Serum protein electrophoresis (SPE) and immunofixation is commonly used to screen for plasma cell dyscrasias. Interpretation of these tests is qualitative by nature and can yield trace, faint, or scarcely visible immunoglobulin bands (TFS), which can be difficult to classify. Whether these bands should be reported at all is challenging given their unknown clinical significance.

In the present study, we retrospectively analyzed 14,036 physician-ordered protein SPE and immunofixation electrophoresis (IFE) tests on serum and urine specimens (from 4,091 patients) during the period of 2000-2010. We found that 17% of all IFE results evaluated for the presence of monoclonal gammopathies (2,389 out of 14,036) contained TFS bands, representing 4.2% (173 out of 4091) of all patients evaluated. Sixty of these patients (42%) had no previous history of gammopathy, and were clinically evaluated over a mean period of up to five years from the original diagnosis of plasma cell pathology.

None of these patients had progressed to multiple myeloma, lymphoplasmacytic lymphoma, plasmacytoma, or leukemia. The remaining 82 patients (58%) had a previous history of gammopathy, but had not progressed to any symptomatic plasma cell dyscrasia. Evaluation of these patients was followed for a median period of 4.3 years, with a mean of 21.5 IFE tests per individual.

These data suggest that for patients without a previous history of gammopathy, the presence of TFS bands on serum protein electrophoresis does not warrant frequent follow up investigation as commonly practiced. Routine follow up of patients with a prior history of gammopathy, conversely, are warranted and may contribute to overall survival with multiple treatment options now available. For those interpreting IFE results, it may be worth considering these data when composing comments regarding suggested repeat testing frequency by SPE/IFE or alternate test methods.

INTRODUCTION

Serum protein electrophoresis (SPE) is commonly used as a diagnostic tool to screen for plasma cell dyscrasias including multiple myeloma, macroglobulinemia, and amyloidosis[1]. Screening by SPE is then commonly followed by immunofixation electrophoresis (IFE) to confirm and classify the specific paraprotein present (IgG, IgA, IgM, IgD, or IgE). Interpretation of both SPE and IFE by trained reviewers is qualitative by nature. In our experience, both SPE and IFE assays can yield faint, or scarcely visible immunoglobulin bands that may be equivocal and difficult to interpret. While these patterns technically fall in the category of "monoclonal gammopathies of undetermined significance," they are truly at the threshold of visual detection and subject to intense debate amongst those who review them daily. We've previously identified, as others have, that this threshold commonly varies from reviewer to reviewer to some extent[2].

Ultimately, the debate centers on whether these faint / equivocal bands should or should not be reported and whether they are of clinical significance. If they are not clinically relevant, they may be the cause for ongoing testing that may extend, on average, 15-20 years beyond initial diagnosis[3], which could prove costly and potentially require unnecessary labor, analysis, and patient risk. If they are of clinical relevance, then they should be identified so that patients may be appropriately monitored, diagnosed, and treated as necessary. Thus, the present study was conducted to determine the clinical significance of these trace or equivocal bands and their correlation to the development of monoclonal gammopathy.

MATERIALS AND METHODS

Study design

This retrospective study included data from 14,036 serum and urine specimens (from 4,091 patients) analyzed by the McLendon Laboratories at the University of North Carolina (UNC) Hospital for physician-ordered protein SPE and immunofixation electrophoresis (IFE) tests during the period of 2000-2010. The subjects' ages ranged from (31 to 90; mean = 62±0.9 years of age) (Table 1). Forty-four percent of subjects were female with 61% Caucasian and the remaining 39% comprised of African-Americans, Hispanics, Asians, Native-Americans, and other races. This study was reviewed and approved by the UNC at Chapel Hill School of Medicine Human Research and Ethics Board (IRB No. #11-0639).

Table 1 **Demographic and racial distribution of patients displaying trace/faint/suspicious (TFS) bands during testing**

Race	Patients with TFS bands (%)	Gender (males; females)	Mean age at first diagnosis (yrs)	Patients with unequivocal bands >3 g/dl	Relative risk	Confidence interval	p-value
African American	54 (31%)	32; 22	58±1.5	2 (40%)	0.98	0.92-1.0	0.54
Asian	2 (1%)	1; 1	59±1.4	0	0.85	0.51-1.4	0.54
Caucasian	105 (61%)	57; 48	65±1.1	2 (40%)	-	-	-
Hispanic	6 (3%)	4; 2	58±4.8	1 (20%)	0.85	0.59-1.2	0.37
Native American	2 (1%)	2; 0	45±1.5	0	0.85	0.51-1.4	0.54
Other	4 (2%)	1; 3	58±8.7	0	0.92	0.69-1.2	0.59
Total	**173**	**97; 76 (56% Male)**	62±0.9	**Out of 5 total patients (Group 8)**	-	-	-

The relative risk (and confidence interval) for a given racial/ethnic group to display unequivocal bands > 3g/dl during SPE/IFE analyses are provided. Risk is calculated with respect to the Caucasian group as reference. Of the total number of patients diagnosed with unequivocal bands >3g/dl (N=5), the percentage listed indicates representation from that racial/ethnic group.

Methodology for serum protein electrophoresis and immunofixation testing

Serum protein electrophoresis and immunofixation testing was performed using the Sebia Hydrasys System (Norcross, GA) or the Beckman Coulter Paragon Electrophoresis System (Brea, CA), according to the manufacturer's directions, as previously described[4-7].

Of the 173 IFE gels identified as having trace, faint, suspicious bands, thirteen were assessed on the Beckman Paragon (used 1990-2002), 160 were run on the Sebia Hydrasys II (2003-2011), and one was run on an unknown system in 1989 (records of the technology used are not available).

Quantitation of monoclonal immunoglobulins

Monoclonal immunoglobulins were quantitated using a combination of the serum total protein (Vitros® 5600 - Ortho Clinical Diagnostics;

Total Protein dry slides, which rely on the biuret reaction) and the % area of the abnormal band determined by scanning densitometry (Sebia, Phoresis Software). At UNC, it was determined experimentally that 0.3 g/dL was the threshold for reproducible, accurate, quantitation of the monoclonal protein using serial dilution of 3 different patient's monoclonal immunoglobulins (undiluted concentration was >5 g/dL). If a monoclonal immunoglobulin was determined to be below 0.3 g/dL, it was not quantitated and defined as *"too low to quantify."* Monoclonal proteins in the alpha and beta regions, or in the presence of a high concentration of polyclonal immunoglobulins were reported on a case-by-case basis.

Inclusion and exclusion criteria

The study focused on identifying patients that had very low concentration abnormalities in serum, as outlined in Figure 1. For inclusion in the present study, both SPE and IFE results were required. In addition, serum immunofixation interpretations had to contain one or more of the following descriptive key words: *Trace; Faint; Suspicious; Possible; Small; Questionable; Equivocal; "?Ig"; Band of restricted mobility; Suggestive of;* and *Weak*. These are heretofore referred to as trace/faint/suspicious (TFS) bands. We excluded urine electrophoresis results, those with a monoclonal protein >0.3 g/dL, results without preceding or subsequent tests, and those without any abnormalities. Any results designated with TFS nomenclature that satisfied the exclusion criteria were also omitted.

Figure 1 shows the study design and inclusion/exclusion criteria. The first row, all protein electrophoresis tests, represents every available SPE or UPE result for a 5 year time period. To define the relevant data, UPE results were excluded (Figure 1, row 2) as were results that indicated a quantifiable monoclonal gammaopathy (Figure 1, row 3). Results were also excluded where there was an absence of serial results, abnormalities, or unequivocal bands (Figure 1, row 4). The remaining dataset included 434 results from 173 unique patients, which were subsequently classified into group (described below).

Classification of patients with TFS bands

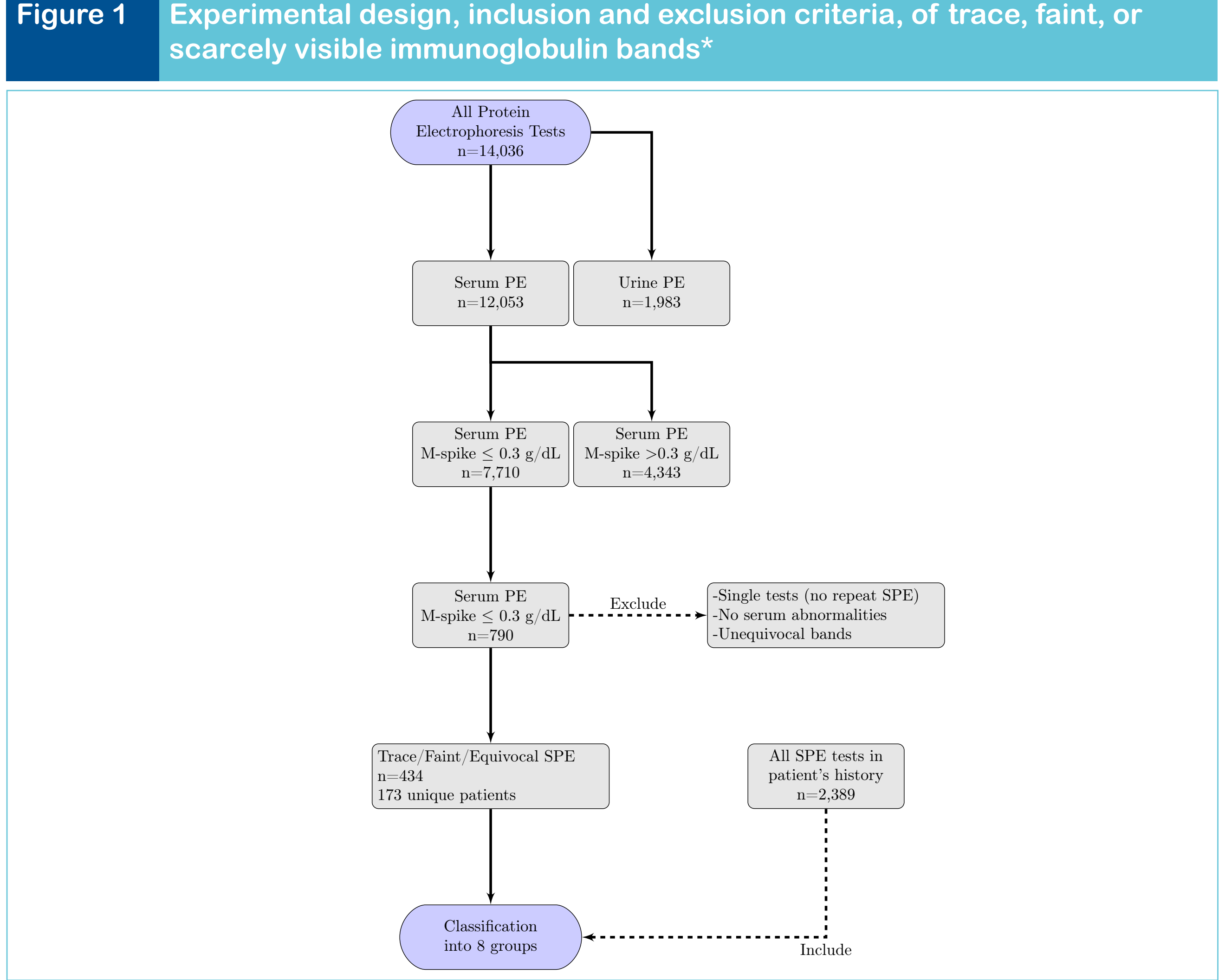
Results that met the inclusion criteria (N=434) were categorized into Groups (I – VIII) as defined in Table 2. Groups are based on the clinical history and concentration of preceding and subsequent monoclonal abnormalities by IFE at UNC that were mined from the SOFT electronic laboratory system; follow up and history of patients were determined by both the laboratory SOFT and the hospital electronic medical record (WebCis). The data include 173 distinct patients. In order to evaluate the clinical significance of the TFS bands in these patients, the complete electrophoretic history from the SOFT electronic laboratory system and the hospital electronic medical record of each patient was included in the final analysis, yielding a total of 2,389 tests.

RESULTS

We identified that 17% of all IFE results assessed at UNC Hospitals and evaluated for the presence of monoclonal gammopathies (2,389 out of 14,036) contained TFS bands, representing 4.2% (173 out of 4091) of all patients evaluated. These patients were further subdivided into two groups based on previous history of gammopathy. In all, 82 patients had an IFE result classified as having a TFS band and history of gammopathy; 60 patients had an IFE result classified as TFS without a history of monoclonal gammopathy.

All patients with TFS bands were sub-grouped according to the concentration of any previous or subsequent abnormal bands. The characteristics and outcomes of each group are indicated

Figure 1 **Experimental design, inclusion and exclusion criteria, of trace, faint, or scarcely visible immunoglobulin bands***



analyzed by the McLendon Laboratories at the University of North Carolina Hospital for physician-ordered protein SPE and immunofixation electrophoresis (IFE) tests during the period of 2000-2010.

in Table 2 and Table 3. Patients in Groups I to IV had no prior history of gammopathy. Patients in Group I had a TFS band, but no subsequent abnormality by IFE, while those in Group II did have a subsequent TFS band or unequivocal band that remained below the detection limit (<0.3 g/dL). Patients in Groups III and IV had subsequent unequivocal bands that were quantifiable at concentrations of <0.3g/dL and >0.3g/dL, respectively. In Group I patients, serial testing ceased after a single normal IFE test, whereas patients in Group II had repeat SPE/IFE tests or were no longer followed due to insufficient clinical evidence for a monoclonal gammopathy. None of these patients had a record of progression to myeloma or had an IFE test with an unequivocal band as of their most recent available record. Similarly, no patients in either Groups III or IV progressed to myeloma as of the most recent report. One individual in Group IV progressed to a "low-intermediate" risk category, according to 2010 International Myeloma Working Group guidelines (patient with just one risk factor for progression, in this case the monoclonal gammopathy)[8]. Patients in Groups V-VIII all had a previous history of monoclonal gammopathy, and were

Table 2 Group characteristics of serum protein electrophoresis testing with trace/faint/suspicious (TFS) monoclonal protein

Group	History	Description	Subsequent gammopathy [concentration]	Prior gammopathy [concentration]
I	No history of gammopathy	Subsequent testing yielded no serum or monoclonal protein		
II		Subsequent testing yielded TFS bands with ultimately no unequivocal, quantifiable band	Trace, Faint, Suspicious Bands	
III		Subsequent testing yielded TFS with ultimately no unequivocal, quantifiable bands	<0.3 g/dL	
IV		Subsequent testing reveal unequivocal monoclonal bands that were quantifiable	>0.3 g/dL	
V	Previous history of gammopathy	Previous serum PE with unequivocal bands that are not quantifiable		<0.3 g/dL
VI		Previous serum PE with unequivocal, quantifiable bands		0.3 to < 1.5 g/dL
VII		Previous serum PE with unequivocal, quantifiable bands		1.5 to <3.0 g/dL
VIII		Previous serum PE with unequivocal, quantifiable bands		>3.0 g/dL

classified according to the concentration of monoclonal protein detected in previous IFE tests. Patients in Group V had unequivocal bands that were not quantifiable (i.e. <0.3 g/dL). Patients in Groups VI, VII, and VIII displayed unequivocal bands that were quantified between 0.3 to 1.5 g/dL, 1.5 to 3.0 g/dL, and > 3.0 g/dL, respectively. Patients from Group V will require further monitoring over time to determine the significance of the unquantifiable TFS bands, and the probability of progression to myeloma. Likewise, patients from Groups VI and VII

Table 3 **Retrospective summary of group outcomes of patients with trace/faint/suspicious (TFS) monoclonal protein**

Group	Mean age at initial testing (years)	No. unique patients	Median no. serum PE/IFX	Median testing period (years)	Results of additional testing/summary outcome
I	63.0±5.1	8	3 (3 to 5)	5 (0.8 to 5.0)	Testing ceased after the first normal serum PE
II	63.2±2.2	35	2 (2 to 13)	0.72 (0.1 to 7.4)	Patients are still monitored with repeat serum PE or are no longer followed due to insufficient clinical evidence. None of these patients have a record of progression to myeloma or have ever had a serum PE that resulted in an unequivocal band as of their most recent record.
III	64.3±2.9	12	5 (2 to 13)	2.66 (0.2 to 6.4)	Nome of these patients has progressed to myeloma as of their most recent record.
IV	71.2±4.4	5	13 (5 to 29)	5.5 (3.9 to 7.4)	No patients progressed to myeloma. One patient progressed to a “low-intermediate” risk category (according to 2010 IMWG guidelines)
V	59.2±1.3	60	14 (2 to 84)	3.4 (0.1 to 11.0)	Require further evaluation to determine the significance of the unquantifiable TFS bands, and the probability of progression to myeloma.
VI	58.4±3.4	11	15 (3 to 56)	4.4 (1.4 to 9.5)	Require further evaluation to determine if the TFS band is a result of post-treatment remission for myeloma or is of no clinical significance.
VII	60.2±7.6	6	26 (12 to 69)	5.5 (2.3 to 14.7)	Require further evaluation to determine if the TFS band is a result of post-treatment remission for myeloma or is of no clinical significance.
VIII	60.6±4.7	5	31 (12 to 80)	3.8 (0.8 to 13.2)	Displayed unequivocal protein bands indicative of multiple myeloma and will require further evaluation to determine clinical significance.

Groups defined in Table 2; additional testing obtained by retrospective chart review.

will require further monitoring to ascertain if the TFS band is a result of post-treatment remission from myeloma, or if significant risk of persistent or returning myeloma exists.

Patients with unequivocal monoclonal protein > 3.0 g/dL (Group VIII) and a previous history of gammopathy (and treatment) have an increased risk of developing multiple myeloma,

despite improvements in therapy options and increased survival[9, 10]. These five patients must be closely followed with on-going SPE/IFE to determine the clinical significance of the present TFS band findings, and the probability of progression to multiple myeloma.

DISCUSSION

In the present study, we evaluated the significance of TFS bands on serum IFE tests from patients being investigated for plasma cell disorders. Of the 12,053 IFE tests assessed, 434 (4%) had TFS bands (M-spike >0.3 g/dL), representing 142 distinct patients. Sixty of these patients (42%) had no previous history of gammopathy, and were clinically evaluated over a mean period of up to five years from the original diagnosis of plasma cell pathology. None of these patients had progressed to multiple myeloma, lymphoplasmacytic lymphoma, plasmacytoma, or leukemia as of their most recent record. The remaining 82 patients (58%) had a previous history of gammopathy, but had not progressed to any symptomatic plasma cell dyscrasia. In the case of patients with previous tests displaying M-spikes > 3.0 g/dL, there is a higher risk of development of multiple myeloma, but no evidence that this has yet occurred. Patients in this latter group have undergone evaluation for a median period of 4.2 years, with a mean of 21.5 IFE tests per individual.

Serum and urine protein electrophoresis are the front line tests used for workup of any suspected plasma cell dyscrasia. They require a balance between clinical sensitivity and specificity so as to not miss patients with disease, but not over-diagnose those who are healthy or who have a benign condition. While the consequences of identifying an isolated monoclonal gammopathy in a healthy individual are generally low, there is some patient risk for unnecessary bone marrow biopsy and additional blood draws over the ensuing years. There is also an associated cost to continuous follow-up to both the patient and healthcare system. The objective of this study was to provide some information as to the clinical progression of patients with very faint abnormal bands identified by serum IFE by assisting the practicing electrophoresis results interpreter in putting their cases into context. The debate between different interpreters seeing or not seeing a particular abnormal band is likely to continue, but evidence from the current study supports that very low concentration bands are unlikely to progress to anything associated with a symptomatic disease state in a short time period (<5 years). Of course, one must consider other testing modalities, such as serum free light chains, and the rare aggressive plasma cell clone. Low concentration B-cell clones may produce toxic monoclonal immunoglobulins that cause severe tissue damage despite being difficult to detect by routine electrophoresis testing[11]. Examples of these include light-chain amyloidosis, light-chain deposition disease, and monoclonal cryoglobulinemia, which may cause irreversible tissue damage. Likewise, it is reported that multiple myeloma can occur despite a scarcely detectable monoclonal component[12]. Collectively, these data suggest that repeat serum IFE testing in patients with TFS bands may not be informative in the short run. In cases where there are TFS bands, but remaining concern for disease, it is probable that other testing modalities, such as serum free light chains and urine protein electrophoresis are appropriate.

All of the patients in this study of low concentration bands effectively meet the criteria for *monoclonal gammopathy of unknown significance* (MGUS). MGUS is defined by production of small amounts of monoclonal protein (< 3g/dL) in the absence of symptoms of myeloma, such as renal insufficiency, hypercalcemia or bone lesions that may be attributed to plasma cells

pathologies. MGUS is significantly more common than myeloma and the incidence increases with age, affecting ~3% of individuals aged 70 and older[13]. Though MGUS is believed to be a pre-myeloma condition, not all patients with MGUS develop myeloma. About 30-40% of individuals with MGUS, given sufficient time, may progress to myeloma, with the risk of progression approximated at 1% per year[3]. The objective of this study was to examine the progression of patients that are at the border of no monoclonal abnormality and MGUS. Given that plasma cell dyscrasias are a continuum from MGUS to myeloma, it is plausible that patients with very low concentration abnormalities are at risk for progression. In the available time frame (median 5 years), it appeared that none of the patients progressed. Based on the 1% progression rate reported by Kyle et al.[3] for MGUS, we might expect that 1-2 of the 142 patients included in this study might progress to myeloma per year. While more time is needed to monitor these patients, it is possible that lower concentration abnormalities are at lower risk for progression. Indeed, some of these low concentration abnormalities failed to persist over time let alone progress. It is unknown whether the TFS bands identified represent a pre-malignant state at all or some other immunological process entirely, such as a targeted immune response.

Marked differences in the incidence of MGUS and multiple myeloma have been reported in both African-Americans and Africans compared to Caucasians (see recent review Greenberg et al., 2012)[14]. The prevalence of MGUS in African-Americans has been reported to be higher than that of Caucasians in patients residing in North Carolina. Cohen et al, (1998) studied 1,732 subjects >70 years of age and found that 8.4% of African-American patients had a monoclonal protein compared to 3.8% of white patients ($p<0.001$), increasing in prevalence with age and greater in men vs. women[15]. In the present study, 31% of patients with a previous history of gammopathy and the presence of TFS bands were black, compared to 61% white patients. Indeed, the percentage of patients with unequivocal bands >3 g/dL were comparable between African-Americans (40%) and whites (40%), despite the lower representation of African-Americans in this study (Table 1). Twenty percent of Hispanic patients displayed quantifiable bands in this category, with no observations for unequivocal bands for Asian, Native American or other patients. Thus, in addition to age, the race of the patient should play a factor concerning the decision to pursue further testing, and determining the likelihood for progression to malignancy, particularly for African-American patients.

For those individuals with a previous history of gammopathy and with serum monoclonal protein >3 g/dL, *smoldering multiple myeloma* (SMM) becomes an important consideration. Similar to MGUS, SMM is characterized by plasma cell proliferation, and is asymptomatic with respect to end-organ damage, but presents with serum monoclonal protein levels higher than that of MGUS. Patients diagnosed with SMM are believed to have a higher incidence of progression to multiple myeloma than those with MGUS, accounting for 10-15% of new cases of multiple myeloma; however, the risk of progression declines significantly over time[3], therefore requiring a shorter period of post-evaluation from the initial determination.

Only some patients with clearly identifiable MGUS will progress to the stage of multiple myeloma. In a study of 241 patients diagnosed with MGUS, 64 individuals (26%) eventually developed multiple myeloma, amyloidosis, Waldenström's macroglobulinemia, immunoglobulin light chain amyloidosis, or some lymphoproliferative disorder[3]. However, these patients were further observed for a median period of 10.4 years before

being diagnosed for a proliferative cell disorder. Similarly, in a larger, population-based study in Minnesota, 115 of 1,384 patients (8%) diagnosed with MGUS and followed over a median period of ~15 years progressed to a proliferative cell disorder[16]. The median age at initial diagnosis of MGUS was 72 years old[16].

Though little is known regarding the mechanism by which progression to a plasma cell malignancy occurs, certain conditions have been suggested as predictive of progression. The following have been reported to correlate with an increased rate of malignant progression from MGUS: serum monoclonal protein values > 2.5g/dL; the presence of an IgM or IgA monoclonal protein; having more than 5% bone marrow plasma cells; an abnormal free light chain ratio[3]. For SMM, progression to malignancy may occur progressively, with gradual increases in serum monoclonal protein levels (termed evolving SMM), or abrupt increases in monoclonal protein values (non-evolving SMM). The rate of progression to malignancy is dependent upon the number or proliferation rate of circulating plasma cells[17].

Despite extensive analysis over a prolonged period, this study has a few limitations that should be considered when interpreting the data. The study includes a maximum of 10 years of follow up for any given patient (median follow-up was 4 $\pm$ 0.4 years) such that the time frame may be too short to identify patients that progress to symptomatic disease slowly. The analysis did not include serum free light chains or urine protein electrophoresis data except where available for the 142 patients included; serum protein electrophoresis was unavailable for the first several years of the time included in the study. Lastly, we have previously reported on the variability between interpreters and methods, which could factor into the classification of results by IFE[4]. Three different platforms were used over the course of the study data and at least 8 different interpreters reported results over the ten-year period.

Collectively, the data in this study suggest that for patients without a previous history of gammopathy, the presence of TFS bands on serum protein electrophoresis does not warrant frequent follow up investigation as commonly practiced (Groups I-IV in the current study). Routine follow up in those with a prior history of gammopathy, conversely, are warranted and may contribute to overall survival with multiple treatment options now available. For those interpreting IFE results, it may be worth considering these data when composing comments regarding suggested repeat testing frequency by SPE/IFE or alternate test methods.

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Organization of the POCT unit

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ABSTRACT

Point-of-care testing (POCT) has evolved as an important part of laboratory medicine by virtue of its compactness, portability, and the feasibility of operation by nonlaboratory personnel, where fast and accurate testing methods are a primary concern and, as a result, improving the patient care. To successfully achieve POCT quality in networks, a multidisciplinary organizational approach is required. A clearly defined organizational structure should be put in place for proper functioning and optimum utilization of each POCT unit. The POCT unit must include designated authority, responsibility, and accountability.

ORGANIZATION OF THE POCT UNIT

Point-of-care testing is also known as extra-laboratory, alternate site, or near-patient testing. It typically refers to the performance of a diagnostic laboratory test outside of a traditional central laboratory and near the site of patient care, whether it is inpatient settings or outpatient clinics. The worldwide volume is rapidly expanding, with a 12% to 15% annual growth rate. The point-of-care testing (POCT) has evolved as an important part of laboratory medicine by virtue of its compactness, portability, and the feasibility of operation by nonlaboratory personnel, where fast and accurate testing methods are a primary concern and, as a result, improving the patient care.(1) The reasons for performing tests in this setting include convenience to the clinicians, a faster turnaround time (TAT), and advantage to the hospital administration in terms of cost savings. Concerns that have arisen with the POCT include problems with ensuring quality, potential conflicts of interest, and an uncertainty of the responsibility.(2) In larger networks, testing may be performed at locations ranging from the emergency room (ER), the operating room (OR), or intensive care units (ICUs) in the hospital to satellite outpatient clinics. The large majority of clinical staff members involved in POCT are focused primarily on clinical care and are much more variable in their familiarity with the testing process and quality control requirements. Training and ongoing competency maintenance of the staff performing POCT can be overwhelming to manage. POCT implementation requires a systematic approach, which involves all stakeholders.

An example of an organizational chart for a POCT network is shown in Figure 1.

Figure 1 Organizational chart for a POCT network

Traditionally, POCT was unregulated and little effort was made by hospitals to establish an approach to managing these technologies. Predictably, issues concerning the quality of test results became apparent, resulting in the establishment of regulatory requirements that have been enforced by hospital accreditation organizations. Failure to comply with regulatory mandates may have major consequences. The need to address regulatory requirements combined with rapid expansion of POCT technologies has resulted in a concerted effort by institutions to manage POCT as a formal hospital program. The first step in developing a strategy to manage POCT involves setting up an interdisciplinary POC management team, including the laboratory, physicians, and nurses.

Tasks and responsibilities can be moved across traditional territories. By identifying where the process crosses territories, opportunities for cooperating and adopting a total system perspective can lead to powerful new solutions to common problems. Typically, the cross-territory concept occurs in three areas of the hospital, i.e. the clinical user unit, the laboratory and the information technology department (ITD). (3) In order to improve the utilization of POCT and the quality of patient care, and to ensure that results are integrated into and being networked with the laboratory information system, the establishment of new relationships among the laboratory, clinicians and the ITD people is needed.(4)

POCT STAKEHOLDERS PLANNING PROCESS

Prior to purchase of POCT equipment, it is recommended that all those with involvement in POCT, namely the POCT stakeholders, are part of the planning process. This group (in a hospital or clinic setting) might include, in addition to a POCT specialist, or specialist in the area under consideration for testing, representatives from the following groups:

- End Users (to state their needs and wants);
- Biomedical engineering (information on analyser design issues);

Table 1 **Organizational structure for small set up (5)**

POCT performed on Hospital Campus	Lab Director
	Technical Consultant
	On-Site Supervisor
	Testing Personnel
POCT in Physician Offices and Clinics	Lab Director
	Clinical Consultant
	Technical Consultant
	On-Site Supervisor
	Testing Personnel

- Information Technology staff (advice on software interfaces and functionality);
- Organisational Quality staff (registration, certification requirements);
- Purchasing / contracts officer (optional, depending on local practice);
- Representative from local pathology laboratory.

Lab director

All POCT to be put under the direction, authority, jurisdiction and responsibility of the Chief of Pathology and Laboratory Medicine.

Responsibilities of the Director of the Clinical Laboratory:

- Responsible for all POCT activities on the SFGH campus;
- Ensures compliance with all applicable regulations, rules and standards;
- Provides expert advice and information to the POCT Committee, including identifying alternatives to various POCT methods and devices, determining criteria for medical necessity, and identifying procedures for adopting and implementing the tests.

Director of the clinical laboratory or an authorized representative (6)

a. Screens, recommends and approves all instruments, devices, procedures, reagents, materials and kits used in POCT, including new lots of previously approved reagents, and supplies, and new versions of any established POCT.

b. Establishes and regularly reviews procedures for all approved POCT, including guidelines appropriate quality assessment processes.

c. Collaborates with the Medical Staff, Nursing Services, and the Department of Education and Training, in the training of individuals designated as trainers and supervisors of the personnel selected to perform POCT.

d. Conducts periodic reviews of POCT performance by monitoring for compliance to established guidelines and providing, as required, proficiency test specimens to each site authorized by the Clinical Laboratory to perform POCT.

e. Conducts periodic inspection of the POCT sites for compliance with regulatory mandates.

f. Periodically reports on the status and performance of POCT as requested by SFGH oversight committees, including, but not limited to PIPS and MEC.

g. Monitors utilization of all POCT, communicates with each POCT site, and recommends methods to improve efficiency to the unit performing the tests and to the POCT Committee.

h. Maintains a current master list of all POCT sites and the types of tests performed at each site.

POCT coordinators as leaders (7)

POCT should be introduced in a systematic process which is inclusive of all stakeholders. Ad hoc approaches are potentially expensive and dangerous in terms of patient safety. To avoid this situation, a POCT Coordinator should be employed.

The POCT Coordinator should be an experienced medical technologist/scientist from a hospital, laboratory or specialist POCT service provider background. The responsibilities of the POCT Coordinator includes overall supervision and management of the POCT activity, ensuring compliance with the policies and quality standards required by the program particularly in relation to selection and evaluation

of instruments, staff training and competency assessment, surveillance of the entire testing process, quality control and quality assurance procedures and resolving technical problems. The POCT Coordinator provides invaluable leadership in collaboration with nurses and physicians. Institutions should set up POCT committees to oversee the POCT service. The committee should be responsible for evaluating and prioritizing new tests and balancing use of limited resources. With numerous tests available for PCT and each year, needs arising for more the POCT committee carefully determines clinical indications, test clusters, and valid applications before approving near-patient or bedside testing.

There will be many people involved in the creation, implementation and management of a POCT service. It is vital that an appropriate senior professional is identified to act as a 'POCT co-ordinator' and given the authority and overall responsibility for the service at the beginning of the development process. This individual will have responsibility for both the results that are generated and the correct use of the devices that generate those results. Managers of POCT should also be aware of their responsibility for clinical governance and of the medico-legal implications of an erroneous result. Liability under the Consumer Protection Act will only remain with the manufacturer or supplier if the user can demonstrate that the equipment has been used in strict accordance with the manufacturer's instructions.

Responsibilities of the POCT coordinator (8)

- Ensuring that all POCT is performed to the same standard as would be expected from regular laboratory testing.

Table 2	Role of the POCT coordinator (8)
	Identifying suitable POCT equipment for evaluation
	Performing an evaluation
	Installing POCT equipment
	Writing procedures
	Training staff
	Preparing worksheets, log books, etc.
	Maintenance schedules
	QC programs
	Trouble shooting
	Monitoring and review of procedures
	Competency reviews

- Identifing the types and locations of all POCT equipment within the CDHB. An electronic record of all equipment is maintained.
- Ensuring that all of the CDHB staff performing POCT have current competency training and documentation, including an awareness of health and safety issues pertaining to samples and equipment.
- Ensuring regular Quality Assurance is maintained and Quality Control (QC) samples are analysed on POCT devices, with up-to-date documentation and history.
- Troubleshooting of POCT devices, with up-to-date documentation and history.

Role of the local hospital pathology laboratory

The local hospital pathology laboratory should play a key role in the development and management of a POCT service. This is particularly true for secondary care and may also be useful for some primary care services. The pathology laboratory can provide advice on a range of issues including the purchase of devices, training, interpretation of results, troubleshooting, quality control, quality assessment and health and safety. There should therefore be close liaison between users and the local hospital pathology laboratory on all issues relating to POCT. Wherever possible this liaison should be formally defined e.g. by a service level agreement specifying the range of products, services, operational details and the responsibilities of the central laboratory and the POCT user

Establishment of a POCT committee (7)

In addition to the appointment of a POCT co-ordinator, the establishment of a multidisciplinary POCT committee to oversee POCT whether in the hospital setting or in some elements of primary care is recommended as good practice. All stakeholders should be represented in a POCT committee e.g. laboratory staff, clinicians, nursing staff, specialist nurses, pharmacists, IT and finance. POCT in the community requires similar stakeholder representation; input from a clinical scientist or a biomedical scientist may be helpful.

The role of the POCT committee may include the following:

- Determining if POCT is justified at a particular location. This would include a clear demonstration of increased clinical effectiveness
- Establishing a system for the continuing audit and assessment of POCT
- Ensuring that no POCT device is used unless it has been looked at by the POCT committee
- Setting up a quality hierarchy to ensure that there is a direct link between the person performing the analysis and the POCT committee
- Establishing the presence of a link nurse or other healthcare professional at the point of service delivery
- Including representatives from primary care and the community where necessary
- Ensuring that users have documented training in the use of POCT devices and that they are fully aware of all contra-indications and limitations
- Ensuring that internal quality control (IQC) and external quality assessment (EQA) schemes are applied to POCT in the same way as they would be for the established laboratory service.

Clinical consultant

Physicians and Nurse Practitioners within the office or clinic serve as a liaison between the laboratory and its clients in reporting and interpreting results.

Technical consultant

Hospital Laboratory is responsible for technical and scientific oversight of the POCT.

Pharmacy and materials management

a. Notifies the Clinical Laboratory upon the arrival of new lots of specified POCT reagents, devices, kits and supplies (e.g., occult blood cards, glucose strips, urine dipsticks, urine pregnancy test kits, etc).

b. Notifies the Clinical Laboratory upon receipt of any shipment of specified POCT materials, supplies or devices.

c. Provides periodic information on utilization and consumption to the Director of the Clinical Laboratory or authorized representative.

On-site supervisor

Nurse Directors, Nurse Managers, and Administrative Clinical Leaders qualify as supervisors based on their education. They will be responsible for the day-to-day supervision or oversight of personnel performing POCT and reporting test results. A supervisor must be accessible to testing personnel at all times testing is being performed.

Responsibilities (6)

1. All POCT devices adopted will: (7)
    - Be used in accordance with manufacturer's or suppliers instructions.
    - Be subject to regular maintenance as specified by the supplying manufacturer.
    - Details of maintenance performed, faults and corrective action taken, will be documented.
    - Only be used for the purpose it has been evaluated and procured for.
    - Be approved for use by the appropriate local management who will be accountable for the integration of the POCT service into the established POCT quality assurance and operational infrastructure.
2. Internal Quality Control will be performed according to the test specific Standard Operating Procedure (SOP) supplied by the laboratory POCT team
3. Electronic rather than visually–read devices will be used whenever possible. (It is recommended that where such visual read devices are in use, the results are checked by at least two trained members of staff).
4. Only trained, certified and competent staff will use POCT equipment. The training and certification of staff will be arranged in conjunction with the laboratory POCT team.
5. All reagent/cartridge/consumable lot numbers will be recorded to facilitate patient tracking in the event of product recall. A direct link must be maintained between patient demographics, test result, and reagent identification numbers.
6. All adverse events relating to POCT will be reported back to Trust Risk Management Team and the POCT committee by local POCT service users using the Trust incident report form. The POCT committee will have the authority to withdraw or suspend service in the event of a safety-related or performance issue or lack of clinical or cost effectiveness.

Responsibilities of POCT users (6):

- All staff must use the equipment in a safe and responsible manner.
- All staff must have a unique operator ID.
- No operator ID must be shared with another staff member.

- An accurate and up-to-date maintenance log for the POCT equipment must be maintained, signed and dated as required.
- All staff members must satisfy the quality control (QC) requirements pertaining to the specific instrument.
- All patient and QC results must be documented. Included with the results should be the operator's initials and the date and time of the test.
- All staff members operating POCT equipment will have up to date competency records.

CONCLUSIONS

To successfully achieve POCT quality in networks, a multidisciplinary organizational approach is required. A clearly defined organizational structure should be put in place for proper functioning and optimum utilization of each POCT unit. POCT unit must include designated authority, responsibility, and accountability. Standard operating procedures and a POCT quality program should be developed and carried out in all areas.

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

Knowledge on the theory of biological reference values in Latin America

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biological reference values, education, knowledge, Latin America

LETTER

In the last years, during my teaching activities in Mexico, Colombia and Chile I have had the opportunity of carry out several small surveys about the knowledge of theory of biological reference values (1,2) using only a question about a very basic concept from this theory:

"Generally, when a biological quantity is measured in a healthy person, what is the probability, expressed in per cent, that the value obtained be out of the corresponding biological reference interval?"

The question, with a voluntary and anonymous answer, that always I repeated using different words and giving all kind of clarifications, has been asked to 146 university graduates directly related to clinical laboratory sciences (also known as laboratory medicine or clinical pathology) working in different cities of Mexico, Colombia and Chile. From the people surveyed, 41.1 % of the answers have been correct [probability = 5 %] and 58.9 % incorrect.

Under my point of view, as foreign lecturer and consultant in clinical laboratory sciences, these results, keeping in mind the simplicity of the survey question, deserve corrective educational actions. It should be taken into account that, although most of Latin

American clinical laboratories biological reference values are neither produced by the own laboratory nor adopted after their validation (as in most countries in the world), the clinical laboratory university graduates must be able to advise physicians and surgeons about the appropriate use of biological reference intervals, including the limitations of such intervals (3).

I hope that most university graduates related to clinical laboratory sciences in Latin America correctly answer the question asked in the survey. If not, the necessity to reinforce the education on biological reference values theory would be more evident.

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

Software review: a new database on the "Effects on Clinical Laboratory Tests" is now available

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Disclosure:
The database reviewed is not a product of Bio-Rad Laboratories.

SOFTWARE REVIEW

The American Association for Clinical Chemistry (AACC) in cooperation with the publisher John Wiley and Sons, Inc. (Wiley) built a new database on the information on interferences/effects of various laboratory tests due to drugs, diseases, herbs and the issues of the pre-analytical phase. AACC Effects on Clinical Laboratory Tests: Drugs, Disease, Herbs and Natural Products is an update of the major reference works edited by Dr. Donald Young:

- Effects of Drugs on Clinical Laboratory Tests;
- Effects of Disease on Clinical Laboratory Tests;
- Effects of Herbs and Natural Products on Clinical Laboratory Tests (co-edited with S. Narayan, PhD).

They are now available in an electronic format. As a promotion Wiley offers a free trial phase so that users may learn more about the useful database before they make a decision to buy it. Here is the link: http://clinfx.wiley.com/aaccweb/aacc/. The presentation, how the database can be used, will be done by using the analyte creatinine and a possible interference caused by acetaminophen.

Page 1 **Login into the database – enter your e-mail address and a password**

AACC EFFECTS ON CLINICAL LABORATORY TESTS Drugs, Disease, Herbs & Natural Produc

WILEY

Login

Email :

Password :

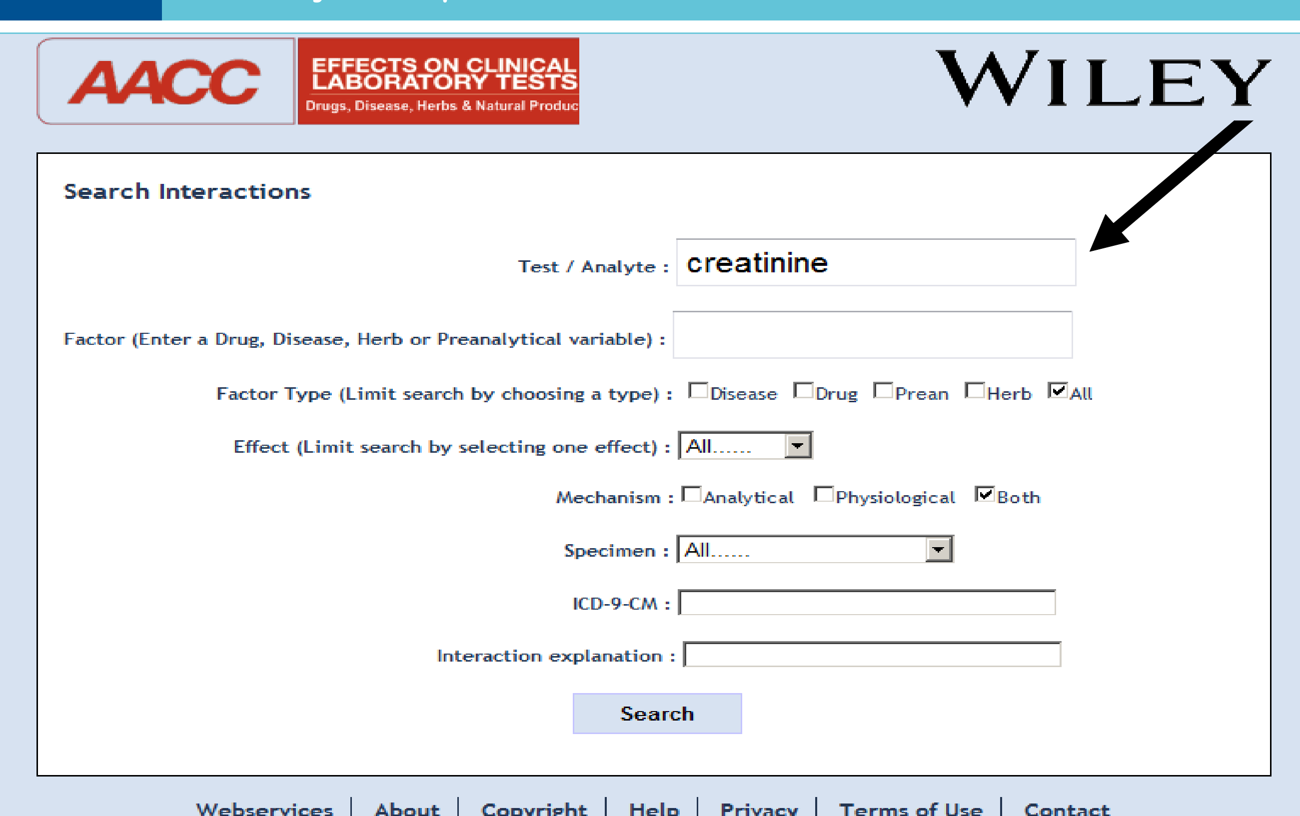
Login

Forgot Password?

Register **here** for trial access.

Webservices | About | Copyright | Help | Privacy | Terms of Use | Contact

Page 2 **Selection – in our example creatinine was chosen as the analyte which may have a possible interference**



Page 3 Selection of possible effects – in our example "acetaminophen"

AACC EFFECTS ON CLINICAL LABORATORY TESTS Drugs, Disease, Herbs & Natural Produc WILEY

Query : Test = Creatinine

1162 factors found

Icodextrin (2)	Colchicum (1)	Intraindividual Variation (22)	Positive Predictive Value (1)
α-Ketoglutarate (3)	Colistimethate (1)	Intralipid (6)	Postpartum (1)
ß-Hydroxybutyrate (4)	Colistin (4)	Iodide (2)	Potassium (3)
γ-Globulin (1)	Congenital Adrenal Hyperplasia (1)	Iodine (1)	Potassium Oxalate (4)
1,2-Diaminopropane (1)	C...ective Tissue Disease (1)	Iodixanol (3)	Potassium Salts (1)
ACE Inhibitors (5)	Contact with Clot (1)	Iodoalphionic Acid (1)	Pravastatin (3)
Abacavir (1)	Coronary Angiography (1)	Iohexol (2)	Prazosin (1)
Acamprosate (1)	Coronary Artery Disease (4)	Iopamidol (4)	Pre-eclampsia (19)
Acebutolol (2)	Corticosteroids (1)	Iopanoic Acid (3)	Prednisolone (4)
Acetaminophen (24)	Cortisone (1)	Iopentol (1)	Prednisone (11)
Acetazolamide (3)	Creatine (12)	Ioversol (1)	Pregabalin (1)

Page 4 After clicking on "acetaminophen" one gets the following information and can refer to the original paper via PubMed

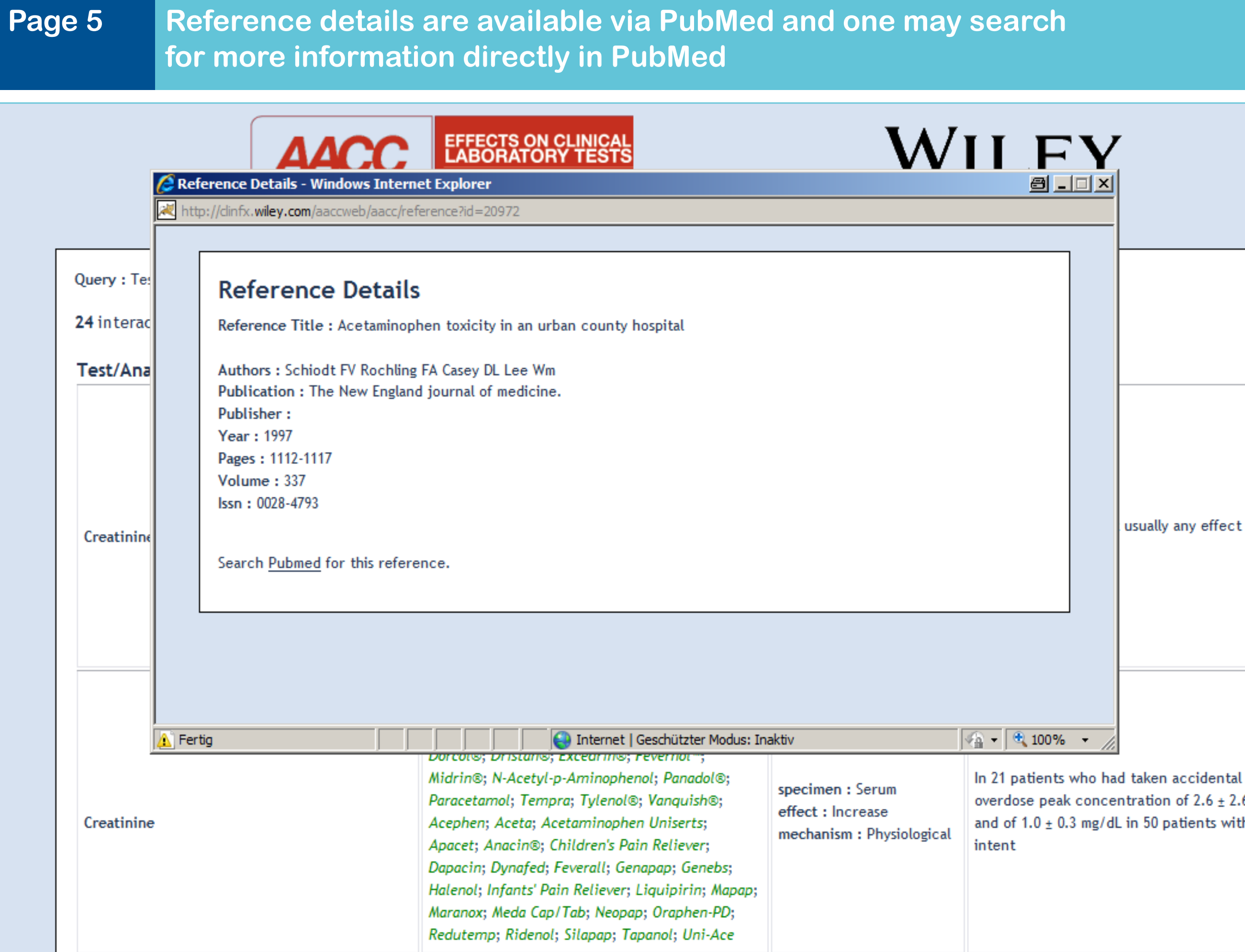
AACC EFFECTS ON CLINICAL LABORATORY TESTS Drugs, Disease, Herbs & Natural Produc WILEY

Query : Test = Creatinine Factor = Acetaminophen

24 interactions found

Test/Analyte Name	Factor Name	Other	Explanation	Reference
Creatinine Clearance	Acetaminophen *syn : APAP; Acetylaminophenol; Anacin-3®; Dorcol®; Dristan®; Excedrin®; Fevernol™; Midrin®; N-Acetyl-p-Aminophenol; Panadol®; Paracetamol; Tempra; Tylenol®; Vanquish®; Acephen; Aceta; Acetaminophen Uniserts; Apacet; Anacin®; Children's Pain Reliever; Dapacin; Dynafed; Feverall; Genapap; Genebs; Halenol; Infants' Pain Reliever; Liquipirin; Mapap; Maranox; Meda Cap/Tab; Neopap; Oraphen-PD; Redutemp; Ridenol; Silapap; Tapanol; Uni-Ace*	specimen : Urine effect : No Effect mechanism : Physiological	Without overdose not usually any effect	Analgesic nephropathy: a reassessment of the role of phenacetin and other analgesics PubMed Search
Creatinine	Acetaminophen *syn : APAP; Acetylaminophenol; Anacin-3®; Dorcol®; Dristan®; Excedrin®; Fevernol™; Midrin®; N-Acetyl-p-Aminophenol; Panadol®; Paracetamol; Tempra; Tylenol®; Vanquish®; Acephen; Aceta; Acetaminophen Uniserts; Apacet; Anacin®; Children's Pain Reliever; Dapacin; Dynafed; Feverall; Genapap; Genebs; Halenol; Infants' Pain Reliever; Liquipirin; Mapap; Maranox; Meda Cap/Tab; Neopap; Oraphen-PD; Redutemp; Ridenol; Silapap; Tapanol; Uni-Ace*	specimen : Serum effect : Increase mechanism : Physiological	In 21 patients who had taken accidental overdose peak concentration of 2.6 ± 2.6 mg/dL and of 1.0 ± 0.3 mg/dL in 50 patients with suicidal intent	Acetaminophen toxicity in an urban county hospital PubMed Search

Page 5 **Reference details are available via PubMed and one may search for more information directly in PubMed**



If you would like to have further information on the database do not hesitate to contact me. Screenshots of "Effects on Clinical Laboratory Tests" are reproduced by permission of John Wiley and Sons, Inc., on behalf of the American Association for Clinical Chemistry.

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