PERSONAL VIEW

A clinician's interpretation of apparently unexplained associations in prostate cancer aetiology: testosterone, anaerobes, Sun deficiency, lack of circumcision, Proliferative Inflammatory Atrophy

Abstract:

Long-term sun exposure, but not spot levels of Vitamin D, reduces risk of Prostate Cancer (PC) occurrence and death. It has been hypothesised that this association is explained by 20-40 years of Vitamin D deficient macrophages facilitating low grade pathogens to induce Proliferative Inflammatory Atrophy (PIA), a known PC precursor. Acquisition of puberty associated infection with sun responsive anaerobe Propionibacterium.acnes (PA) is a possible contributor that could also explain reduction by long-term sun exposure. The observation that circumcision reduces anaerobes on the glans penis (and presumably spread via anoxic urethra to prostate) could explain reduced PC (OR 0.86) in circumcised men. Benefits of circumcision were higher when combined with religious hygiene rules (OR 0.25). GlobalCan data, suggesting that long-term genital hygiene had equal impact to surgery, added support to this view**.** Observed association of reduced testosterone levels with high Gleason grade and over expression of androgen receptor, suggest that andropausal decline of T could be a major factor in cause of late age of PC deaths in absence of PSA screening. Large scale studies of androgen replacement in andopausal men and developing therapies to reduce inflammation related PSA positivity could improve the specificity of PSA screening for PC

Introduction: The average age of death of prostate cancer (PC) in the absence of PSA screening is 75, suggesting that age is one of the most significant aetiological factors in this condition. This is part of a group of observations that are raising queries about the importance of testosterone in development of clinically significant PC. The others are firstly the fact that high grade tumours present with a higher degree of testosterone deficiency than low grade tumours (1). As a result of this, when PC patients are tested at diagnosis, overall PC populations do not have higher testosterone or dihydrotestosterone levels than matched controls (2), though adolescents from populations with greatest risk do have (3). Secondly bone scanning of patients without metastasis demonstrates that PC patients show a higher degree of osteoporosis than matched controls without PC (4). The third observation supporting a lesser role of testosterone in clinically significant PC is that patients with hormone refractory Prostatic cancer treated with testosterone 10 of 27 showed a short-lived reduction of PSA averaging 24% (5) and a similar proportion of such patients if given chemotherapy after stopping androgen blockade subsequently responded a second time to retreatment with androgen blockade (6). In addition when androgen blockade is used intermittently a substantial minority of patients can have recovery of testosterone and lack of progression (7, 8)

During the last two decades searches for prostate cancer genes has dominated investigation into the aetiology. Though evidence for at least 40 low-penetrance genes is now established (9) , the lack of gene environmental interaction studies for most of them (apart from those involved in inflammatory pathways (10) means their practical value is limited at present (11) except in prognostication (12). Dietary factors (13) such as high fat diet, high red meat and low fruit and vegetables (particularly Carotenoid derived Lycopenes) has also dominated research for some time, culminating in the recent publication incriminating a variety of processed meats though not clarifying what process is specifically involved (14) . One other life-style factor that has dominated research in the last decade is exercise and physical fitness Though this has mostly involved study of exercise before the diagnosis, more recent

studies have suggested that those who do regular exercise after diagnosis may have a survival advantage equivalent to that of chemotherapy in patients with end stage disease (15). As, until recently, most exercise is done outdoors, the effect of sunshine induced Vitamin D increase as a confounding variable in exercise studies has to be considered given recent observations of increased vitamin D levels in those who exercise regularly (16).

The suggestion that Vitamin D might be a significant factor in Prostate Cancer came from early studies on the effect of geography through latitude leading to reduced incidence in countries with high sun exposure (17). Subsequently case control studies showed that PC patients had less frequent exposure to regular sunshine (18). Despite these strongly positive associations, multiple studies of impact of a single estimation of serum Vitamin D level failed to correlate with risk of prostate cancer occurrence (19), possibly because opportunistic PSA screening diluted the population with non-lethal prostate cancer or the sun has other cancer prevention benefits such as melatonin (20) or Nitric oxide induction (21) . The dilution hypothesis could explain the few studies that have demonstrated a correlation with serum Vitamin D have been those that have studied the association with advanced disease and death from PC. A final possibility for the stronger association with imprecise indices of sun exposure rather than spot serum levels of Vitamin D would suggest that it is due to long term Vitamin D deficiency possibly over 10-40 years as a possible explanation. Most studies of the role of Vitamin D deficiency in causation of cancer have studied its role as a regulator of calcium metabolism and cell differentiation (16). Much less attention has been paid to its potential role through Vitamin D deficiency leading to deficiency of cathelolithcidin production by Macrophages in response to chronic infection (22). Though so far only studied in Tuberculosis, were Cathelithicidin's non-antigen specific macrophage mediated host surveillance mechanisms to be equally important in other chronic infections (23), deficiency could allow persistence of low grade pathogens to set up chronic inflammation that could lead to Proliferative Inflammatory Atrophy (PIA) of the prostate, now thought to be an important precursor of PC (24). Despite multiple investigations of sexually

transmitted infections, there has been no consistently associated organism with PC (25). However recently infection with a facultive anaerobic organism, the Vitamin D responsive teenage acne associated Propionibacterium.acnes (PA) has shown a more consistent association with prostate cancer. Recent studies of circumcision in HIV prevention have suggested that circumcision reduces anaerobe colonisation of the Glans Penis (26) which has direct access to the prostate through the anaerobic urethra (27). Though anaerobes have long been associated with prostate cancer (28) and antibiotics known to clear them , few have considered them causative. Because of a long held view that a "Helicobacter-like" process could be involved in causation of Prostate Cancer (29-31) this paper updates this hypothesis and considers how this new information contributes to the 40-60 year life history of development of lethal prostate cancer and what experiments and new clinical studies are required to firm up this interpretation and develop a more rational approach to prevention of prostate cancer and improve specificity of PSA screening

The Yin/Yang roles of testosterone in prostate cancer development

Today it is increasingly accepted that PSA screening works to an extent and that prostatectomy in early cases saves some lives (32). It undoubtedly leads to the diagnosis of patients with prostate cancer 10-20 years prior to clinical presentation and the patients so detected have less advanced tumours with fewer metastases . However the actual survival benefit is small and has to be set in context of the reduction in quality of life for eight to ten years before the benefits begin to accrue. This small gain also has to be set against the excess of over diagnosis and treatment of tumours compared to the numbers presenting clinically and the large numbers of individuals with elevated PSA who have no cancer on biopsy and have prolonged uncertainty of its significance. These factors led the US Preventative Services Task Force in 2011 to conclude that PSA screening "……has no net benefit" (33) though greater attention to reducing over-treatment of low grade tumours and improved screening methods has somewhat improved the therapeutic benefit (32) A similar

over diagnosis and false positive rate is seen with mammography screening for breast cancer (34) and cervical smear based screening for cervix cancer (35). It is thought that this excess could represent tumours that are controlled by immune surveillance under normal circumstance and in the case of cervical cancer actually undergo spontaneous regression and the frequency of this could be increased by use of condoms by male partners (36). Though there is continuing debate as to how much of the benefit from breast screening is actually due to mammography (37), there is less anxiety about over-diagnosis of cervix cancer, because, compared to radical prostatectomy or radiotherapy to the prostate, diathermy of the cervix produces less morbidity. Were it possible to reduce the false positives and reduce the over treatment with a simple less intrusive approach, the cost benefits of PSA screening would improve dramatically.

Since the observations of Nobel Laureate Charles Huggins , testosterone has long been assumed to play a significant role in PC aetiology. The observations of Ross et al that teenage Africans when compared to European or Japanese teenagers had higher levels 5 alpha reductase that increased the intra-prostatic androgen Dihydro-testosterone (3) might in part explain the African's later higher incidence of PC. This led to development of the 5 alpha reductase blocking drugs as possible agents for chemo-prevention of prostate cancer. Though successful, in reducing low grade tumours, both drugs appeared to increase the risk of high grade tumours (38, 39). The observations of Ross et al were nevertheless important for another reason in drawing attention to hormone levels at puberty because at that time authors had begun to raise the possibility that prostate cancer had its origins in puberty with reports that an early age of onset of sexual intercourse was associated with increased risk of prostate cancer (40) though subsequent studies only demonstrated its influence in 5 of 8 studies where it had been studied (41). Three observations provide a possible explanation for this divergent view on the role of androgens at puberty and in later life. Firstly several systematic reviews of the correlation of late adult life levels of Testosterone and Dihydro-testosterone showed less significant association with PC risk (2) than Ross et al's studies in

teenagers (3) . Secondly patients presenting with high grade more lethal PC tended to be older and have lower testosterone at presentation than those with low grade tumours (1) suggesting that the andropause may contribute to generation of high grade tumours. Thirdly testosterone supplements in patients with hormone refractory Prostatic cancer treated with testosterone 10 of 27 showed a reduction of PSA averaging 24% (5) and a similar proportion of such patients if given chemotherapy after stopping androgen blockade subsequently responded a second time to retreatment with androgen blockade (6). Fourthly patients with pre-cancer of the Prostate have been given Testosterone replacement without causing progression to advanced cancer

Interaction between Testosterone and innate immune surveillance

The observations that the imprecise indices of sun exposure (table 1&2) rather than spot serum Vitamin D levels (42) seem to suggest that early in adult life, sun induced Vitamin D deficiency (or some other as yet to be discovered sun-induced immune-regulatory molecule (43)) combined with high intra-prostatic dihydrotestosterone could facilitate colonisation of prostate by low grade pathogens beginning around puberty. An example of such an organism is PA (a known sunshine sensitive organism frequently causing skin problems around puberty), which is increasingly been found in human prostatic cancers (44, 45) and in animal models being shown to initiate the inflammatory response found in human tumours (46). As it has not been possible to consistently show a single organism linked to prostate cancer (25) , it is likely that it is the multiplicity of insults contributing to the inflammatory milieu that is the critical issue and prolonged period of exposure.

The John Hopkins's group have given the lead in studying the role of inflammation in development of prostate cancer. They compared histological changes in the prostate in the non-malignant and malignant areas of prostates removed surgically for cancer. They demonstrated a high prevalence of proliferative inflammatory atrophy (PIA) that they

hypothesised occurred as a consequence of repeated slow healing of low grade prostatic inflammation attacks whether auto-immune or infectious (24). This condition consisted of focal atrophic lesions (which develop following acute/chronic inflammation) within which, there is an increased fraction of proliferating cells, compared to normal or atrophic epithelium seen in the ageing prostate. These atrophic lesions were first described by Franks in 1954 and in 1996 Feneley et al demonstrated an increase in Ki67, a marker for mitosis, in association with these atrophic lesions. However the idea has become increasingly mainstream when the Baltimore Group showed increased evidence of PIA in patients with Prostate intra-epithelial Neoplasia (PIN) as well as prostate cancer. (24) In addition they demonstrated in families with prostate cancer that there was linkage with defective anti-viral, macrophages and Cox-2 genes (10) that might contribute to an increased propensity to excessive inflammatory response.

In this respect the data on PA (Table 3) could be significant. As a known facultive anaerobe, Propioinibacter acnes could also explain the link with an increase risk of PC associated with lack of circumcision (Table 4&5) in view of the demonstration of accumulation of anaerobes under the foreskin by Price et al (26). These could presumably track up the anoxic urethra to colonise anaerobic scars from aerobic infection. That circumcision may not be the sole factor is suggested by the difference between religious and other context of surgery (Table 4) and by Globocan data (Table 5) which shows similar incidence in Circumcised Bangladesh and mainly uncircumcised India and Circumcised USA and Uncircumcised Denmark. The decreased frequency of circumcision in African Americans (47) and increased protection it gives to those who undergo circumcision (48) would suggest that this could be a possible confounding contributor to their increased risk. Over the course of multiple decades of urban living, such anaerobes organisms as PA could facilitate PIA development possibly in association with development of an autoimmune type of reaction (24), something which would be facilitated by prolonged periods of low sun exposure. However it is declining testosterone in old age that is the final promoter of

development of the high grade tumours with selection of genes switched onto cell cycle proliferation (12) and androgen resistance with over expression of the androgen receptor from this semi-autonomous PIA to produce the clones that kill. This hypothesis helps provide an explanation for one other unexplained association with development of prostate cancer, ie declining ejaculation (49) and raises the question whether Oxytocin (50) (also suggested to reduce breast cancer (51)) and/or PDE 5 inhibitors (52) might have a role in prostate cancer prevention. The observation that breast cancer is increased with early menarche and increased mammary density, a risk factor for breast cancer that is also associated with Vitamin D deficiency (53) suggests that the hypothesis that life-long decreased resistance to low grade pathogens might also be relevant to the aetiology of breast cancer. It might also explain the association of Vitamin D deficiency (54) and deceased intake of dietary fibre with an increase in colon cancer given the colon's high anaerobe content and stem cells that are the fastest dividing cells in colonic Krypts of Lieberkuhn. It might also contribute in part to explain why sun-deficient Scotland has a higher frequency of lung cancer deaths than the sunnier parts of England (55) and why a small study of oral microbiome from patients with proven pancreas cancer have a higher level of anaerobes than controls (56) .

These observations also provide a possible explanation as to why PSA at 45 (57) is a better predictor of PC death at 75 than it is at 65. They also lead to the conclusion that the provocative suggestion of Tannock that PSAitis associated over-diagnosis of prostate cancer that PSA screening induced did more harm than good (58) may not be true if it was possible to find simple treatments that "cured" PSAitis at the age of 45. Such treatments could well substantially improve the predictive value of PSA screening as was first suggested in 1995 (29) and enable radical treatment to be focused on the patients who benefit from early treatment (59) and thus reduce the harms that are increasingly being quantified in those over-treated with latent cancer (60)

What further studies could support these observations and provide opportunities for chemo-prevention of prostate cancer?

The simplest first step would be to attempt to prove that vitamin D deficiency leads to slower healing following episodes of prostatic infection (as measured by the time it takes to return PSA levels to normal). These should preferentially be done with Afro-Caribbean patients as they have more Vitamin D deficiency (61) requiring 6 times the dose of UV to make 50% of the response of a Caucasian (62) and in a study in pregnant women, Africans gained more from Vitamin D supplementation than Caucasians (63)

Studies by Sutcliffe et al. (64) have demonstrated that PSA levels may decline at a slower rate after a prostatic infection than prior to its onset. If healing can be shown to be slower in those with Vitamin D deficiency, a follow up prospective randomized placebo controlled trial of immediate verses deferred Vitamin D supplementation within a cohort of Vitamin D deficient patients who also demonstrate raised PSA levels after prostatic infection, could be conducted to provide important information about this hypothesis.

The second step would be to conduct a study involving men with a raised PSA, detected either as part of a screening trial or in men with proven negative needle biopsy. The study would examine the state of foreskin associated hygiene and anaerobic colonisation of the glans penis (26) as well as in the ejaculate produced by these individuals as P. acnes specific miRNA sequences have recently been demonstrated in pooled semen from prostate cancer patients but not controls with a negative biopsy (65) . If it can be demonstrated that an association exists between anaerobic colonization and a raised PSA level, the next step might be to undertake a trial of antibiotic treatment appropriate for anaerobic organisms, +/- circumcision and examine its effect on PSA levels in these men.

Thirdly, anti-inflammatory chemo-prevention trials could be initiated in men with persistently elevated PSA but negative biopsy, building on the positive trials undertaken with both

Finasteride and Dutasteride (39) . Similar studies would also be possible in those with stable but raised PSA on Active Surveillance or Watchful Waiting. Possible agents worth considering would be a Cox2 inhibitor (66) Vitamin D(or sunlamps to study non-vitamin D effects of sunshine) and the new class of Vitamin D analogues , or even short courses of modern immune enhancing anti-cancer therapy such as the anti-CTLA4 monoclonal antibody, Equimimob (67). Finally, if randomised trials were to precede with second generation HPV vaccines in boys at start of puberty, they should be followed up with studies to assess whether such intervention leads to a lower PSA level at 18 years of age (68) than unvaccinated controls.

Conclusion:

Since the observations of the association of Helicobacter with stomach cancer, the concept of "germ" related inflammation has been a dominant hypothesis for acceleration of tumour mutational evolution for nearly 20 years (69-71), though it has not been seen as such a long process (though similar to the effect of smoking) nor has it been linked to lack of sunshine enhancing bacterial colonisation of anaerobic scars. Lack of association of PC with spot Vitamin D but a stronger association with index of sun exposure suggest that this association could still be due to reduced long term Vitamin D mediated non-specific macrophage surveillance or some other sun-enhanced resistance against lowly pathogenic agents of which PA, could be a lead organism. Studies, particularly in urban living Africans with both a high incidence of Vitamin D deficiency and Prostate cancer, which investigate the impact on PSA of elimination of these organisms could become an important strategy in future efforts of to improve the specificity of PSA screening and chemoprevention of PC

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Table 1

Long term sun exposure index and Prostate cancer incidence

	No of cases	OR (High vs. Low sun exp)
Luscombe CJ, 2001 (18)	210	0.75
Bodiwala D, 2003	212	0.75
John EM, 2007	160	0.59
Gilbert R, 2009 (72)	986	0.76
Nair-Shalliker, V, 2012	1,084	2.07
Chia, S-E, 2012	240	2.03

Table 2

Correlation of Prostate cancer incidence in high vs low sun regions

Authors	Population	Correlation
Hanchette & Schwartz 1992 (17)	3073 US counties	0.19
Freedman DM, 2002	97, 873	0.9
John & Schwartz 2004	3414	0.68
Schwartz & Hanchette 2006	17,536	0.27
Boscoe & Schymora 2006	3.1×10^6	0.8
Lagunova & Moan 2007	46,205	1.01
Grant 2010	French population	0.64
Loke 2010	70 LGA (median 133,765)	0.52

Lin SW, 2012	21,439	0.91

Table 3 **Propionibacterium.acnes and prostate cancer**

	Organism	Number of individuals	Risk
Galobardes 2005	P. acne history & PC death	11,273	OR 1.67
Sutcliffe 2007 (44)	P. acne antibiotic use	16 vs 2071	OR 1.7
Alexeyev 2006	P. acne 16S RNA	171 vs 181	OR 2.17
Severi 2010	P. acne antibody titre	809 vs 504	OR 0.73
Fassi Fehri, 2011 (73)	P. acne Imunoflourescence ab	71 vs 29	82% vs 21%

Table 4

Correlation of Circumcision Status and Prostate cancer occurrence

(for references see (47) (74) (75)**)**

Non-religious Circumcision studies	No of controls	No of cases	OR (CI)
TOTAL	**2803**	**2750**	**0.87, (0.76-1.00)**
Benign prostatectomy	**Non Jewish**	**Jewish**	

studies			
TOTAL	**40768**	**2878**	**0.25, (0.1-0.73)**

Table 5

Circumcision & Global Incidence/Mortality of Prostate Cancer (PC) & Cervix Cancer (CC) (GLOBOCAN 2008)

	Deaths PC per 10	**Incidence PC per 10**	**Deaths CC per 10**	**Incidence CC per 10**
Brazil circ-	16.3	50.3	10.9	24.5
USA circ+	9.7	83.8	1.7	5.7
Denmark circ-	2.5	72.5	2.5	11
India circ-	2.5	3.7	15.2	28.9
Bangladesh circ+	1.2	1.9	17.9	27
Saudi Arabia circ+	5.1	7.7	0.9	2.1
Israel circ+	7.6	55	2.1	5.6
China circ-	1.8	4.3	4.6	9.6
Japan circ-	5	22.7	2.6	9.8

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