VASCULAR ENDOTHELIAL GROWTH FACTOR AND PROSTATE CANCER

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Esta dissertação teve como base dois manuscritos, em que fui responsável pela recolha e armazenamento da informação, pela definição das hipóteses em estudo, pela análise e interpretação dos dados que reportam, tendo elaborado as versões iniciais de ambos os manuscritos:

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1.1. PROSTATE CANCER INCIDENCE

Prostate cancer was the second most common cancer diagnosed among men worldwide, only behind lung cancer, representing 12% of all cancers (excluding skin non-melanoma) (1). It was estimated that approximately 679000 men worldwide had a prostate cancer diagnosed in 2002, 76% of these in developed countries, accounting for 19% of all cancers in these settings (Table 1).

In Europe, prostate cancer was the most frequent cancer in men in 2006, with 345900 new cases (20.3% of all cancers, excluding skin non-melanoma), corresponding to an age-standardized incidence rate (European standard population) of 86.7/100 000 men (2). In the United States of America (USA) prostate cancer already represents 25% of all cancers (excluding skin non-melanoma) in men (3). The frequent utilization of PSA testing and the growth in its worldwide use accounts to an increasingly high proportional weight of prostate cancer (4) and to a reduction of the average age at diagnosis (5).

In less developed countries, prostate cancer was estimated to be the sixth most common cancer among men, responsible for 5.3% of all cancer diagnoses in 2002, behind lung (15.5%), stomach (13.1%), liver (11.8%), esophagus (8.3%) and colorectal (6.3%) cancers (Table 1).

Table 1: Prostate cancer frequency.

	Region		
	World	More developed countries	Less developed countries
Number of cases	679023	513464	165347
Percentage of all cancers (excluding skin non-melanoma) among men	12%	19%	5.3%
Crude incidence rate (/100 000 men)	21.7	88.4	6.5
Age-standardized * incidence rate (/100 000 men)	25.3	56.2	9.4

* World standard population

Source: GLOBOCAN 2002 (1)

After allowing for differences in the age structure of the populations, prostate cancer incidence was nearly six-fold higher in the more developed countries (56.2 cases/100 000 men) than in the less developed (9.4 cases/100 000 men) (Table 1). In 2002, the highest country specific age-standardised incidence rate (world standard population; per 100 000 men) was 124.8 in the USA, and the lowest was 0.3, in Bangladesh.

Along with family history, Sub-Saharan African ancestry has long been recognized as a risk factor for prostate cancer (6). In addition, Afro-Americans are more likely to be diagnosed at a younger age and to have more aggressive forms of prostate cancer than European-American men (6). This contrasts with the lower incidence rates observed in Asian men (7). Despite the racial determinants of prostate cancer, much of the geographic variation in its perceived frequency is related to the ability to detect latent prostate cancer (8, 9).

The trends in prostate cancer incidence are also heterogeneous across countries, and largely determined by the generalized increase in the use of transurethral resection of the prostate, since the early 1980s (10), and especially by the widespread PSA testing since the 1990s.

Data from the USA (11), United Kingdom (12) and some Asian countries (13) clearly shows an increase in the incidence of localized prostate cancer since the 1990s, reaching almost the double between 2000 and 2005, while the rate of advanced cancers has steadily decreased up to 30%. Nonpalpable cancers (TNM clinical stage T1c) now account for approximately three quarters of incident cases in the United Kingdom (14). Much of this stage migration has been attributed to PSA testing (15).

Differences in the exposure to environmental risk factors for prostate cancer, namely the dietary, may explain some of the geographic and temporal variation of its incidence (16). Foods containing lycopene or selenium probably protect against prostate cancer (17). An association between calcium intake and the risk of prostate cancer has been reported but the results are not consistent across studies (18). There is limited evidence suggesting a positive association with higher meat intake, milk and dairy products and an inverse associations with intake of plant foods and foods containing vitamin E, namely cereals, soy products, and fruit and vegetable sources of carotenoids (17, 19).

Chemoprevention may also contribute to the differences in prostate cancer incidence and in its time trends across regions, as these drugs are used most in developed countries and with increasing frequency in the last years (20). The results of the study SELECT have now been reported, showing no impact of selenium or vitamin E on the risk

of prostate cancer (21). The results of the PCPT study, testing finasteride, showed a significant 24.8% reduction in the risk of prostate cancer (22). Dutasteride, tested in the REDUCE trial, also reduced the risk of prostate cancer by 23% (23). Other studies are currently ongoing to test the efficacy of other drugs, such as statins, in prostate cancer prevention (24, 25).

Heterogeneity in the completeness of cancer registration may also impact comparisons (26, 27). In Norway, inaccurate reporting of prostate cancer was estimated in less than 1% (28), in a population cancer registry in Saarland (Germany) the estimated completeness was 91% for prostate cancer (29) and the Estonian Cancer Registry had an overall completeness of case ascertainment of 90.8% (30). On the other hand, a hospital cancer registry in Thailand included only 56% of all histologically verified cancer cases (31) and a cancer registry from a major public referral hospital in Saudi Arabia had an estimated ascertainment rates of 51% for all cancers (32). The apparent lower completeness of cancer registries in less developed countries may contribute to the lower official incidence estimates of prostate cancer in these settings. The proportion of incident cancers that only come to the registry's attention via a death certificate notification of cancer also varies across registries. In the case of prostate cancer this varies from less than 1% in USA (0.8%), Switzerland (0.4%), Denmark (0.0%), Sweden (0.0%) and Singapore (0.0%) to higher than 12% in Japan (17.5%), Kuwait (13.6%), Bulgaria (13.0%) and Portugal (12.2% in the Registo Oncológico da Região Sul) (1).

The percentage of cases for which the diagnosis was based upon microscopic verification of a tissue specimen is as an indicator of the validity of the diagnostic information. For prostate cancer it was higher in USA (97.0%), The Netherlands (97.8%), France (98.8%), Pakistan (100.0%) and lower in Bulgaria (62.5%) and China (56.3%) (1). Again, this can justify some of the reported differences between prostate cancer incidence throw the world.

1.2. SURVIVAL OF PROSTATE CANCER PATIENTS

The 5-year age-standardized (world standard population) relative survival estimates for prostate cancer in 2002 were higher among men living in more developed countries (76%) than in those from less developed regions (45%) (26). In Europe the age-standardized (european standard cancer population) average 5-year relative survival for prostate cancers diagnosed between 2000 and 2002 was 77.5%, much higher than the 58% observed in 1988 (33, 34). Nevertheless, these estimates are substantially lower than the 99.3% 5-year age-standardized (international standard for cancer survival analysis (ICSS)) relative survival reported in the USA for cases diagnosed in 2000–2002 (33, 35), although direct comparisons between these regions are limited by the use of different standard populations.

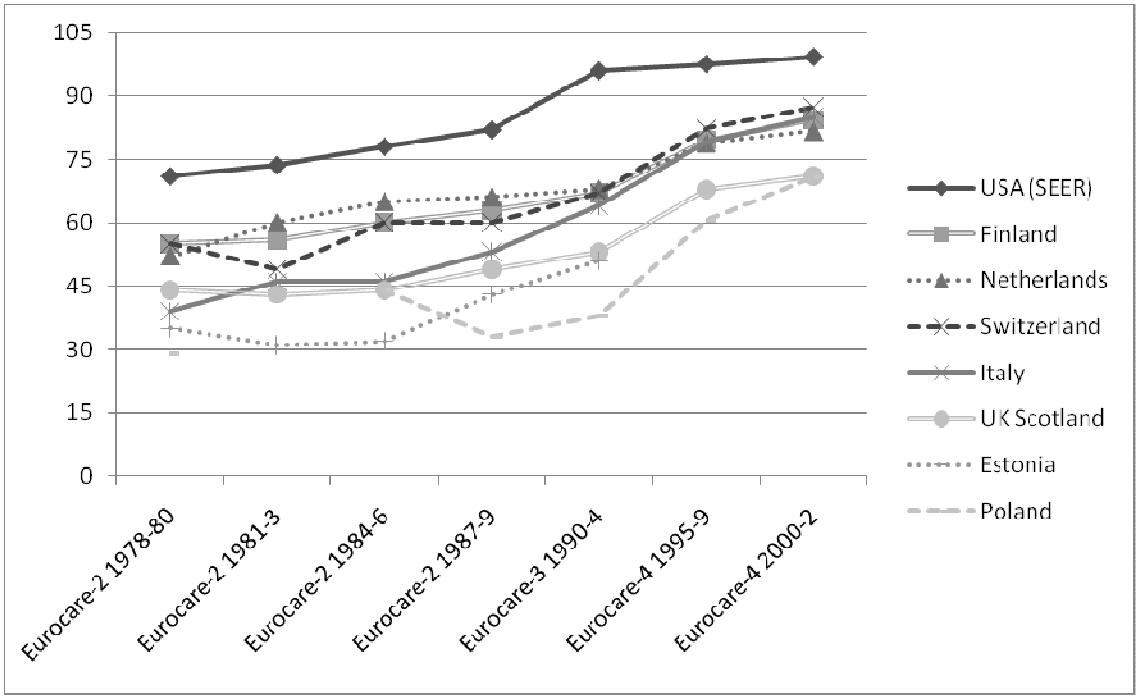
In prostate cancer cases diagnosed in the period 1990-1994, the 5-year age-standardised (ICSS) relative survival was higher than 85% in the USA (91.9%), Canada (85.1%) and Austria (86.1%) and lower than 40% in Denmark (38.4%), Poland (37.1%), and Algeria (21.4%) (36). For the southern region of Portugal the crude 5-year relative survival estimate was 47.7% (95 percent confidence interval (95% CI): 40.7 – 54.8) (36).

The trends in prostate cancer patients' survival are summarized in Figure 1, although it should be interpreted with caution, not only due to intercountry differences in the frequency of PSA testing, but also due to the diverse standard populations used in the different studies.

The widespread PSA testing has led to a migration towards younger age and less advanced stage of disease at diagnosis (15), and currently no statistically significant differences are observed in survival by age-group (42). For localized disease, survival was similar for Afro-American men compared to Caucasians in the USA (6). However, for advanced prostatic cancers survival has been consistently lower among Afro-Americans (6).

The interpretation of prostate cancer survival information also needs to take into account lead-time bias and overdiagnosis. The lead time is estimated to be between 4 and 12 years for prostate cancer, and the detection of cancers, that otherwise would not have been diagnosed within the patient's lifetime, is estimated to be between 22% and 66% of all prostate cancers detected with PSA (8, 43-45). Both can raise artificially survival estimates without reflecting any real clinical benefit, and mortality rates provide more meaningful information regarding the effectiveness of prostate cancer management than survival data alone.

Figure 1: Trends in 5-year relative survival (%), for adults (aged 15–99 years) diagnosed with prostate cancer.



SOURCE: EUROCARE 2 (37, 38), EUROCARE 3 (39), EUROCARE 4 (33, 40) AND SEER (35, 41)

Standard population: Eurocare-2 (1978-89): age-distribution of cases of the overall European sample; Eurocare-3 (1990-4): derived from the age distribution of all patients included in that study for each cancer, and were thus cancer-specific; Eurocare-4 (1995-9) and SEER: International standard for cancer survival analysis (ICSS) that employs standard age distributions that differ according to the age pattern of incidence of the cancer (elderly population in the case of prostate cancer); Eurocare-4 (2000-2): European standard cancer population.

1.3. PROSTATE CANCER MORTALITY

An estimated 221 000 men died from prostate cancer throughout the world in 2002, corresponding to an age-standardized rate (world standard population) of 8.2 deaths/100 000 men (table 2). Worldwide it represented less than 1% of all male deaths and about 7% of all cancer-related deaths among men during 2002 (46). Sixty percent of the prostate cancer deaths occur in the more developed countries, where prostate cancer was the seventh most common cause of death in 2005 (4% of all male deaths), but it does not rank in the top 20 causes of death among males in middle or lower income countries (corresponding to less than 1% of all deaths) (47).

Table 2: Prostate cancer mortality.

	Region		
	World	More developed countries	Less developed countries
Number of deaths	221002	130382	90514
Crude mortality rate (/100 000 men)	7.1	22.4	3.6
Age-standardized* mortality rate (/100 000 men)	8.2	13.5	5.2

* World reference population

Source: GLOBOCAN 2002 (1)

In Europe, prostate cancer accounted for 9.2% of all cancer-related deaths in men, making it the third leading cause of oncological death, after lung and colon/rectum cancers (2). In the USA, mortality was higher in Afro-American compared to European-American (age-standardised (ICSS) mortality rates: 33.0/100 000 men *vs.* 12.6/100 000 men) probably due to higher incidence, more advanced stage at detection and poorer access to health care among the former (35, 48).

From 1979 to 2005, a significant reduction in prostate cancer mortality was observed in most of the more developed countries (11, 49-52). One explanation for the decline in mortality could be the development of more effective treatments, for prostate cancer at all stages (53). New surgical techniques, irradiation protocols and antiandrogenic therapies could have played an important role (54, 55), as well as the improved access to these treatments due to the increased emphasis in the control of prostate cancer (52).

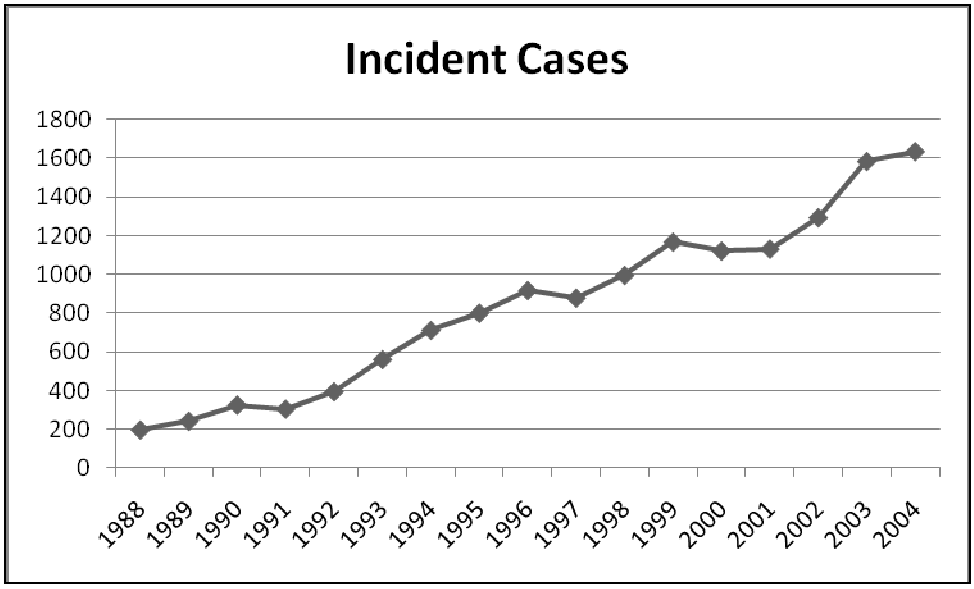
Other probable explanation is that this decreased mortality is the result of improved detection at early stages, mainly due to the increasingly use of PSA (51, 56). This is a very controversial issue as it has not been clearly shown that PSA screening decreases prostate cancer mortality despite recent published trials (57, 58). Besides, some authors have argued that the fall in prostate cancer mortality occurred too soon after the widespread use of PSA (59). It was expected that the decrease would have taken at least the mean lead time of prostate cancer to be apparent. However this could be explained by the hypothesis that PSA screening is effective in reducing mortality only (or mainly) on the fraction of cases with shorter pre-clinical phase (60). On the other hand, no association was found between the intensity of PSA screening and the subsequent reduction in mortality (61).

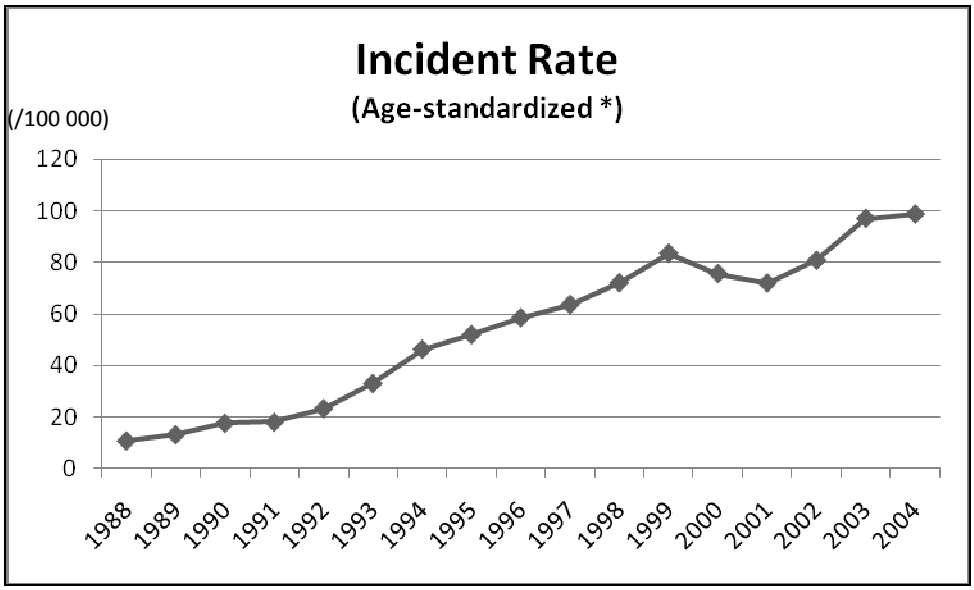
1.4. BURDEN OF PROSTATE CANCER IN PORTUGAL

In Portugal, prostate cancer was the most common malignant tumor in males in 2002, with an age-standardized (world reference population) incidence rate of 46.8 cases per 100 000 men, representing 18.8% of all cancers in men (1).

According to the ROR-Sul, in 2001, prostate cancer incidence was 50% higher in Lisboa and 20% higher in Setúbal districts than in the other regions (62).

Figures 2 and 3 : Prostate cancer incidence trends in the north of Portugal.





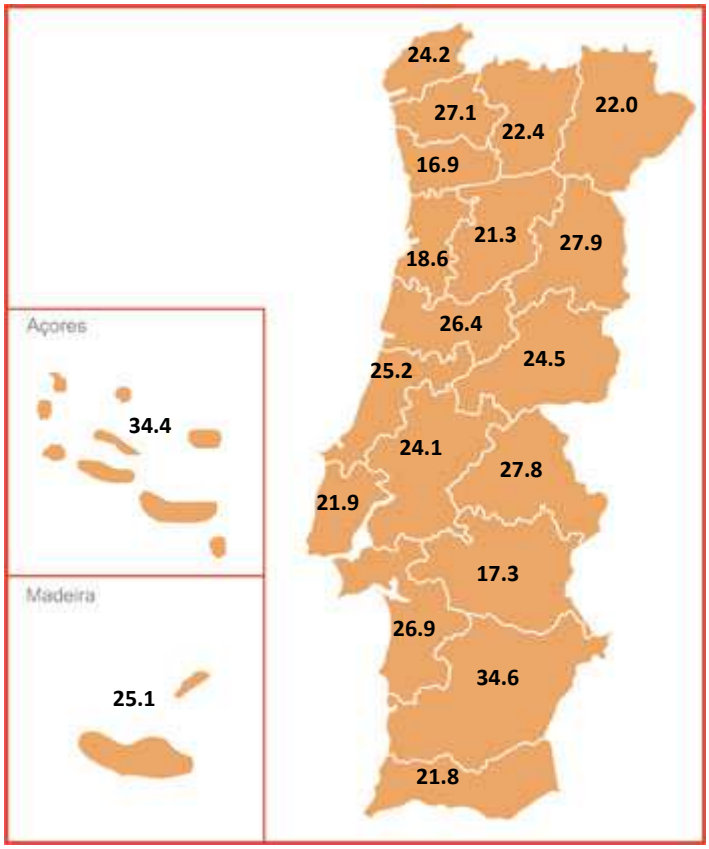
* European reference population

Source: RORENO (63)

Prostate cancer incidence has been increasing steeply in the northern region, with a 8-fold increase in the number of cases and a 9-fold increase in the age-standardized incidence rate from 1988 to 2004 (Figures 2 and 3) (63).

Population-based survival data in Portugal is available only from the ROR-Sul. In this cancer registry, the 1, 3 and 5 year relative survival for prostate cancer patients diagnosed in 2000-20001 period was 94%, 88% and 83%, respectively (62), and regional differences were also observed. Relative survival was significantly higher in the region of Lisboa e Vale do Tejo (95%, 89% and 84%, respectively) than in the remaining regions, the lowest figures being observed in Algarve (87%, 81% and 77%, respectively).

Figure 4 - Age-standardized (European reference population) prostate cancer mortality rate (/100 000), by district and autonomous regions in 2005



Source: Risco de Morrer em Portugal 2005 (64)

In Portugal, prostate cancer mortality was responsible for 1636 deaths in 2005, corresponding to an age-standardized (European reference population) mortality rate of

23.1 per 100 000 men (64). Prostate cancer mortality varies between districts being lower in Porto district (16.9/100 000 men) and higher in the Região Autónoma dos Açores (34.4/100 000 men) (figure 4) (64).

In men older than 44 years prostate cancer mortality increased 1.9%/year between 1980 until 1999, and since then it has been decreasing 3.4%/year at least until 2005 (65). This decrease in mortality was higher in the districts where the rates were also higher in the beginning of that period (65).

2. SCREENING AND DIAGNOSIS OF PROSTATE CANCER

Most prostate tumors arise in the periphery of the gland, distant from the urethra, rarely causing symptoms early in the course of the disease. Symptomatic cancers usually correspond to locally advanced or metastatic disease (66).

The main tools for early detection of prostate cancer include digital rectal examination (DRE), PSA testing, and transrectal ultrasonography. Transrectal ultrasonography has a low predictive value for localized tumors and is not recommended as a screening test (67). Only DRE and PSA testing are used for first-line assessment of prostate cancer risk (68). Serum PSA is a better independent predictor of cancer than suspicious findings on DRE (67, 69). However, DRE allows the detection of 21.0% more cases than PSA alone (using a threshold of 4 ng/mL) (70), and the tests are recommended in combination by the European and American Guidelines for early diagnosis of prostate cancer (68, 71).

The use of PSA testing increased dramatically in the last years (72-74). In the United States of America more than 50% of men older than 50 years and 87% of male physicians had a PSA test between 2001 and 2004 (75). There is some evidence that DRE is performed in less than 50% of the men submitted to a PSA test (76) as many men are uncomfortable with DRE because they are embarrassed or fearful that the examination will be painful (77).

Even with the information of two recently published randomized trials (57, 58), the benefit of screening and early intervention on mortality is still controversial (78-81). However, the European Urology Association and the American Urological Association, recommend that PSA testing and DRE should be offered to men with a life expectancy of at least 10 years for early detection of prostate cancer (68, 71). The American Urological Association updated its guidelines and now even recommends annual screening for men aged 40 and older who have a life expectancy of at least 10 years (71). According to both these entities, the decision to undergo early PSA testing should be a shared decision between the adequately informed patient and his physician.

The diagnosis of prostate cancer is based on histological examination of prostate tissue, derived from prostate biopsies or, occasionally, from surgical specimens collected after prostate benign pathology surgery or other pelvic surgeries. Prostate biopsy should be performed under transrectal ultrasound guidance and a minimum of eight cores obtained (82). The decision whether or not to have a prostate biopsy should be made considering all

the information available for each patient. This includes serum PSA levels, DRE findings, prostate size, ethnicity, age, comorbidities, patient's concern with health, history of previous biopsy, presence of prostatic disease, previous diagnostic procedures and prostate-directed treatments (68, 71, 83).

However, even taking into account all the information available, PSA and DRE testing are unsatisfactory to decide which patients should undergo a biopsy, because they lack both specificity and sensitivity (80, 84). The development of novel biomarkers or imaging modalities, that could further contribute to decide which patients should be referred to prostatic biopsy, is urgently needed to enhance the yielding of screening and early detection (80, 84, 85).

2.1. PROSTATIC SPECIFIC ANTIGEN

Prostate-specific antigen (PSA) is a kallikrein-like serine protease produced almost exclusively by the epithelial cells that line the acini and ducts of the prostate gland. It is secreted in high concentrations into seminal fluid, where it is involved in liquefaction of the seminal coagulum (86), and was first used by forensic scientists as a marker for human semen in cases of sexual assault (87).

PSA is found in low concentration in the serum and it circulates in both bound (complexed) and unbound (free) forms. Cancer cells produce lower levels of PSA than BPH cells but release a greater amount into circulation, likely reflecting both basal membrane disruption and the disordered cellular and glandular architecture of prostate cancer (88). Its existence was first reported in 1970 by Ablin (89) and isolated by Wang (90) in 1981, but it was correlated with prostatic cancer only in 1987 (91). Its generalized use started in the 1990s, first in the United States, then in remaining develop countries and progressively to the rest of the world (11, 13, 92, 93). In the beginning it was used as a marker of disease recurrence after treatment and latter on as a marker prostate cancer risk (87, 94).

There are many different commercial test kits for measuring PSA, but there is no commonly agreed international standard (95, 96). The initial studies on PSA relied on assays standardized to the Hybritech® standard, but in 1999 the Expert Committee on Biological Standardization of the World Health Organization (WHO) introduced alternate reference material for PSA (the so-called 96/670 standard material). Currently, about half of the commercially available PSA tests are standardized to Hybritech® and the other half to the WHO standard. This is relevant as PSA results are approximately 23% lower with the WHO standard (97-99).

Despite the use of PSA as a screening tool is debatable, it increases the detection rates of prostate cancer (94, 100), especially of cancers more likely to be confined, eventually allowing for curative treatment (14, 15), and its widespread utilization drove a stage shift favoring localized disease (101).

The level of PSA is a continuous parameter, directly related to the risk of prostate cancer (102, 103), and there is no universally accepted cut-off (68, 81, 85). The initial commercial test reported the "normal" range as 0-3.99 ng/mL and the first trials used this value as a cut-off (88).

PSA levels can be modified by factors other than prostate cancer. Its serum levels may be elevated in the presence of benign prostatic hypertrophy (BPH), prostatitis, pre-malignant lesions (atypical small acinar proliferation or high-grade prostatic intraepithelial neoplasia), after prostate manipulation or urological trauma (104). They may be lowered by 5-alpha reductase inhibitors, hormonal manipulation, radiotherapy or surgery (86, 105). Other factors like ejaculation, weight, carbohydrate intake, insulin resistance, metabolic syndrome and bicycling are also reported to affect PSA levels but most of these do not have a clinical relevant effect (86). These factors should be taken into account when deciding to perform a PSA test and interpreting its values.

In general, subjects with a PSA level between 3 and 4 ng/mL have a probability of prostate cancer of about 27%, and in patients with a PSA between 1 and 2 the probability is 17% (102). Due to the risk of missing a clinical relevant cancer there is a trend towards lowering this value, with a consequent increase in the number of unnecessary biopsies, and specially overdiagnosis (106, 107).

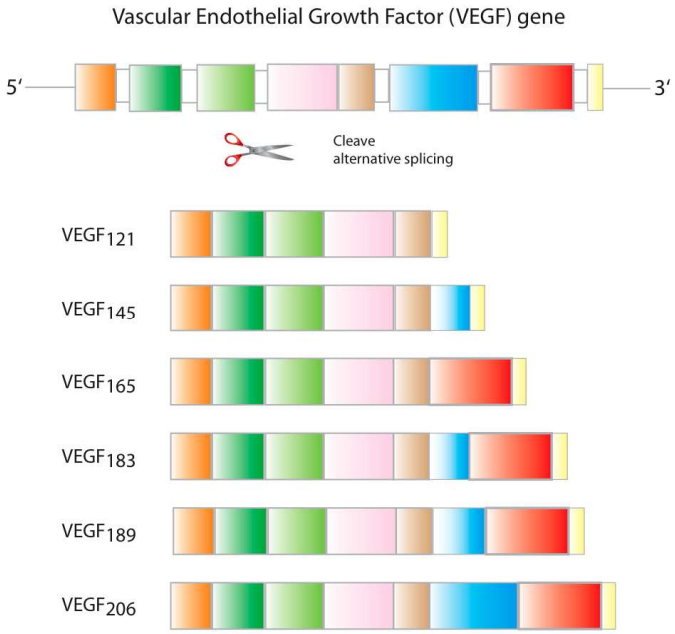
Several modifications of serum PSA value have been described, which improve the specificity and/or sensitivity of PSA in the early detection of prostate cancer. They include adjustment to age and ethnicity, free/total PSA ratio, PSA density, PSA density of the transition zone, PSA velocity and certain PSA isoforms (cPSA, proPSA, BPSA, iPSA). However, all have limited usefulness in the routine clinical setting and a new prostate cancer biomarker that will substitute PSA testing is yet to come (68, 83, 108).

Despite the controversy of the utility of PSA as a screening technique, it´s use in Urology is well establish as a prostate cancer prognosis marker (109, 110) and it has a key role in the follow-up and decision making of prostatic cancer patients after initial treatment (68). PSA levels also correlate with future prostate cancer risk in men without prostate cancer (111, 112).

3. VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular Endothelial Growth Factor (VEGF), also called vascular permeability factor or VEGF-A, is a homodimeric 34 – 42 kDa protein first described in 1983 (113). The gene encoding VEGF resides on chromosome 6p21.3 with a coding region spanning approximately 14 000 bases. The human VEGF gene contains eight exons. At least six isoforms of the protein are found secondary to alternative splicing of the messenger RNA. All six spliced messenger RNAs are homologous in exons 1–5 and in exon 8, but vary in exons 6 and 7 (Figure 5).

Figure 5 – VEGF gene and its isoforms.



The resulting isoforms are named VEGF followed by the amino acid content of the protein: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{162}$, $VEGF_{165}$, $VEGF_{165b}$,$VEGF_{183}$, $VEGF_{189}$ and $VEGF_{206}$ (114, 115). VEGF isoforms are secreted as homodimers of the cysteine-knot superfamily and show similarity to the platelet-derived growth factor family (116). Three isoforms of VEGF ($VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$) are preferentially expressed in VEGF-producing cells (117). $VEGF_{121}$ is readily diffusible but apparently has no important physiological role and $VEGF_{189}$ is tightly matrix-bound due to interactions with heparin

sulfate proteoglycans. The heparin-binding $VEGF_{165}$ is found in circulation and represents the major angiogenic form (116).

In addition to VEGF (or VEGF-A), the VEGF family includes several other greatly structurally related proteins: placenta growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and VEGF-F (115, 118-121). PIGF is primarily expressed in the placenta and its levels are inversely correlated with preeclampsia, but it is also detected in non negligible amounts in the heart and lungs. VEGF-B, which is similar to VEGF in its amino acid sequence (approximately 43% identical), is mitogenic for endothelial cells and can form heterodimers with VEGF, being involved in angiogenesis in the muscles and the heart. VEGF-C and VEGF-D are thought to affect primarily the development of the lymphatic vasculature, through VEGFR-3. Finally, viral homologs of VEGF were described, mainly produce by the orf virus, collectively termed VEGF-E, and a homologue protein isolated from snake venom was designated VEGF-F.

VEGF is considered the most important form in tumor tissues, although other isoforms have been found in some variants of tumors (122). There are three VEGF tyrosine kinase receptors: VEGFR-1(Flt-1), VEGFR-2(FLK/KDR), and VEGFR-3. VEGF also binds to other receptors whose physiological importance is not yet clear (115, 118, 122, 123). VEGF signaling through VEGFR-2 activates several intracellular pathways that ultimately lead to angiogenesis by inducing the proliferation, survival, sprouting and migration of endothelial cells, and also to increased endothelial permeability (124, 125). VEGF gene expression is mainly activated by the transcription complex hypoxia-inducible factor in response to hypoxia.

VEGFR-1 plays a negative role in angiogenesis in the embryo, most likely by trapping VEGF, and a positive role in adulthood in a tyrosine kinase-dependent manner. VEGFR-1 is expressed not only in endothelial cells but also in macrophage-lineage cells, and promotes tumor growth, metastases, and inflammation (124).

Bottom line, VEGF, is a strong inducer of vascular permeability and stimulator of endothelial cell migration and proliferation, and is an important survival factor for newly formed blood vessels (115, 125). VEGF has also been shown to induce lymphangiogenesis (123, 126).

3.1. VASCULAR ENDOTHELIAL GROWTH FACTOR AND CANCER

In adults, physiological angiogenesis occurs almost exclusively in the female reproductive cycle and in wound healing. It also plays an essential role in pathological conditions, such as rheumatoid arthritis, age-related muscular degeneration, proliferative diabetic retinopathy, atherosclerosis, postischemic vascularization of the myocardium and cancer (127).

Cancers are dependent on angiogenesis for growth and spread. In small avascular tumors diffusion is enough to supply oxygen and nutrients, but as the tumor grows it needs increased blood supply via new blood vessels (128, 129). It has been demonstrated in several types of human cancer that tumor dormancy is a function of an impairment in angiogenesis (130). When tumor cells overexpress angiogenic factors or alter the regulation of endogenous angiogenic factors, they develop an angiogenic phenotype, a process referred to as the "angiogenic switch" (128, 129). Based on this property, tumor cells stimulate proliferation and migration of endothelial cells of the host to form an irregular network of vessels that provide nutrients and oxygen (129, 131).

The vascularized tumor has the potential to rapidly expand its cell population and has a propensity to metastasize (128). Accumulating evidence indicates that, for most tumors, the switch to the angiogenic phenotype depends upon the outcome of a balance between angiogenic stimulators, where VEGF plays a central role, and angiogenic inhibitors. Both stimulators and inhibitors can be produced by tumor cells and by certain host cells (128-130).

Tumor lymphangiogenesis actively promotes enhanced draining leading to lymph node metastasis (132). Primary tumors induce new lymphatic vessel growth throw lymphangiogenesis promoters including VEGF, VEGF-C and VEGF-D (133). The remarkable enlargement of sinusoidal lymphatic endothelium might facilitate tumor cell transport to the lymph nodes, and potentially contribute to the migration, residence, and/or survival of metastatic tumor cancer stem cells by inducing a specific tumor microenvironment (132).

VEGF has been shown to be secreted by many solid tumors and tumor-associated stroma in response to hypoxia, inducing a mitogenic response on binding to its receptors in nearby endothelial cells (115). A substantial increase in the microvessel count and VEGF expression in the stage of premalignant lesion was reported for the oral mucosa, uterine cervix, stomach, thyroid, parathyroid and dysplastic nevi (131). Immunohistochemical

expression of VEGF was demonstrated in gastric metaplasia and dysplasia, in atypical adenoma of the colon, atypical hyperplasia and *in situ* breast carcinomas (131).

VEGF serum levels in patients with cervical (134), bladder (135), papillary thyroid (136), kidney (137), pancreas (138), gastric or colorectal cancers (139-141), lymphoma (142), multiple myeloma (143), melanoma (144), cholangiocarcinoma (145), pediatric solid tumors (146) and advanced lung cancer (147) have been reported as significantly higher than in healthy individuals, but not in the case of localized breast (148), localized nasopharyngeal (149), hepatocellular (150) or medullary thyroid carcinoma (151) or leukemia (142) and results were conflicting for ovarian cancer(152, 153). VEGF levels have been correlated with poor prognosis in breast , kidney, brain, cervical and colon carcinomas (123).

Overwhelming evidence has shown that therapeutic interference with VEGF function potently inhibits tumor formation, growth and vascularization. VEGF monoclonal inhibitors or VEGF receptors' inhibitors are currently approved for metastatic breast, colon, kidney, gastrointestinal stromal tumor and lung cancer (115, 118). These drugs are also already been tested in different phases of human clinical trials in advanced head, neck, ovarian, prostate and pancreatic cancers, melanoma and lymphomas (115).

3.2. VASCULAR ENDOTHELIAL GROWTH FACTOR AND PROSTATE CANCER

In prostate cancer, immunohistochemical studies have demonstrated that cancer cells stained positively for VEGF and its receptors, reflecting an increased microvessel density (154-157). VEGF staining and increased vascularity were much higher than in benign prostatic hyperplasia or normal prostate cells. Also, for high grade prostate intraepithelial neoplasia there is a statistically significant increase in microvessel density, VEGF, VEGFR-1 and -2 expression as compared with the observed in normal prostatic tissue (157, 158). Microvessel density was also shown to correlate with higher Gleason grade, pathological stage and the expression of VEGFR-1 and -2 (157).

An unexpected discovery was that androgens regulate VEGF expression, not only in the normal prostate but also in prostate cancer (159, 160). VEGF is also required for the vascular response to androgens and for the ability of the prostate to regenerate in response to androgens (161). Hormone withdrawal, on the contrary, causes a reversal of the neovascularization (162). These data suggest that VEGF, as well as angiogenesis, may play an important role in the early progression of prostate cancer (125). Moreover, VEGF expression in prostate cancer tissue, was shown to be significantly associated with disease aggressiveness (163).

Since the prostate is an important source of systemic VEGF in the case of prostate cancer (164), VEGF is an attractive candidate for a new biomarker to detect patients with high risk of prostate cancer. Several studies have tried to study the association between VEGF and prostate cancer with conflicting results (125).

4. OBJECTIVES

This dissertation aims to address the role of VEGF in clinical practice, in particular in improving the early detection of prostate cancer.

Two investigations were conducted, with the following specific objectives:

- To review systematically the published studies assessing the role of VEGF serum or plasma levels in prostate cancer detection
- To evaluate VEGF as a biomarker for early detection of prostate cancer, comparing its serum levels across groups of patients with suspected prostate cancer, presenting different prostatic pathologies, including benign prostatic hyperplasia, prostatitis, high grade prostate intraepithelial neoplasia (HGPIN) and prostate cancer.

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VEGF and prostatic cancer: a systematic review

Abstract

Introduction: Elevated Vascular Endothelial Growth Factor (VEGF) blood concentration reflects its prostatic production, making this a potentially interesting tumour marker to support the decision of submitting a patient to prostatic biopsy.

Objective: To review systematically the evidence on the role of VEGF blood concentration in prostate cancer detection.

Methods: Published studies addressing the relation between serum or plasma VEGF levels and prostate cancer were identified searching Pubmed®, ISI Web of Knowledge℠, SCOPUS™ and LILACS® up to April 2009, and reviewed following a standardized protocol.

Results: Three studies reported higher plasma VEGF (pg/mL) in patients with localized prostate cancer than in healthy controls (7.0 *vs.* 0.0, 9.9 *vs.* 2.2, and 210 *vs.* 26.5, $p<0.01$), and two showed higher serum VEGF (pg/mL) in prostate cancer patients than in patients with benign prostate hypertrophy (518.9 *vs.* 267.9, $p<0.001$; no specific values, $p<0.05$). In one study, serum VEGF was significantly lower in healthy controls than in patients with benign prostate hypertrophy, localized or metastatic prostate cancer. The only two studies that used controls with previous suspicion of prostatic cancer but a negative biopsy reported non-statistically significant difference in VEGF serum levels (pg/mL) between controls and localized prostate cancer patients (206 *vs.* 241 and 69.5 *vs.* 55).

Conclusion: Higher VEGF plasma levels are observed in prostatic cancer patients compared to healthy controls, but serum levels do not appear useful in differentiating benign from malignant prostatic disease using, as controls, individuals with high risk of prostate cancer and negative biopsy.

Introduction

Prostate cancer is the most frequently diagnosed noncutaneous cancer in men (1). Its incidence increased dramatically after the introduction of the prostate-specific antigen (PSA) test but the lack of sensitivity and specificity of PSA as a diagnostic test is of great concern in clinical practice (2-4). The need for better detection and optimal staging of prostate cancer underlies the need for new biologic markers that could help avoid unnecessary prostate biopsies, allowing the detection of prostatic cancers with low PSA levels (5, 6).

The growth of prostate cancers, as many other solid tumors, depends on angiogenesis (7). Vascular endothelial growth factor (VEGF) is the most prominent cytokine responsible for endothelial cell differentiation, migration, proliferation, tube formation, and vessel assembly (8).

Prostate is a significant source of systemic VEGF in prostate cancer patients (9), as it is synthesized either by adenocarcinoma cells and tumor-infiltrating lymphocytes (10, 11).

Elevated blood VEGF levels could reflect prostatic VEGF production, making VEGF a potentially interesting tumour marker to support the decision of submitting a patient to prostatic biopsy. However, previous reports on this topic provide conflicting evidence.

This study aims to systematically review the published studies assessing the role of serum or plasma VEGF levels in prostate cancer detection.

Methods

Published studies addressing the relation between VEGF plasma or serum levels and prostate cancer were identified in Pubmed®, ISI Web of Knowledge℠, SCOPUS™ and LILACS®, from inception to April 2009. For Pubmed®, ISI Web of Knowledge℠ and SCOPUS™ the search expression was: (Vascular Endothelial Growth Factor OR VEGF OR VEGF-A OR Vascular Permeability Factor) AND (Prostate OR Prostatic) AND (Cancer OR Neoplasm). For LILACS® the search terms were: Vascular Endothelial Growth Factor OR VEGF OR VEGF-A OR Vascular Permeability Factor. Two authors screened the reference lists to identify potentially relevant studies. Papers reporting original research published in English, Spanish, French, Portuguese and Italian were eligible. Papers written in other languages were also considered for the systematic review when the English abstracts provided the necessary information.

Two articles written in Russian (12, 13) were included in the systematic review, but only the information provided in the English abstract was considered after several unsuccessful attempts to contact the authors.

We excluded from the review the studies not conducted in humans, who did not evaluate VEGF-A (the classical VEGF and the most important form in tumor tissues (7)), who evaluated VEGF levels in biological products other than plasma or serum, in whom VEGF was not assessed as a potential diagnosis tool for prostate cancer (*e.g.* measurements done more than one year before diagnosis) or when the control group included cancer patients. The reference lists provided by the identified papers were screened following the same criteria. The systematic review flowchart is presented as Figure 1.

Data extraction was independently conducted by two reviewers following a previously defined data collection protocol. The following information was obtained from each study: year of publication and country of origin, patient selection/study design (consecutive patients, case-control), number of participants diagnosed with the different prostatic pathologies (healthy controls, benign prostatic hyperplasia, localized prostatic cancer, metastatic prostatic cancer or hormone-refractory metastatic prostatic cancer), biologic product used for VEGF evaluation (serum or plasma), method of VEGF measurement (quantifying all the five VEGF-A isoforms or only VEGF-A 121 and 165), VEGF levels in the different prostatic pathologies and corresponding P-values, measure of association and respective precision estimate for the relation between VEGF and PSA levels, biopsy or specimen Gleason score and clinical or pathological staging. Any discrepancies between the two reviewers were resolved by consensus, or involving a third researcher.

No information was available on the method used for patient selection and type of controls from two reports (12, 13), and no description of the method used to measure VEGF and its levels in the different prostatic pathologies could be obtained from one (12).

Due to the limited information provided in the original reports regarding the precision of the estimates and the small number of studies with similar characteristics regarding the control group selection and methods used to quantify VEGF, both potentially responsible for heterogeneity in the observed VEGF levels across studies, no meta-analysis was conducted.

Results

The eight studies considered for systematic review (12-19) are summarized in figure 2 and table 1. Four studies were conducted in Western European countries (3 in the United Kingdom (14, 16, 19) and 1 in France (18)), two in the United States of America (15, 17) and the other two in Russia (12, 13). The median sample size was 92 participants, ranging from 47 to 264.

In four studies (14-17) prostatic cancer cases and controls were selected separately (this study design has been called case-control with two-gate design using healthy controls (20)), one selected a consecutive series of patients undergoing prostatic biopsy (18), and the others used a non-consecutive series of patients with suspected prostatic cancer due to elevated PSA (not further specified) in which the authors choose some patients with BPH, localized prostatic cancer and metastatic prostatic cancer (19).

In three reports controls were men with negligible risk of prostate cancer based on digital rectal examination and PSA levels (14, 15, 17). In two other studies (18, 19) controls were men with high risk of prostate cancer who had a negative prostate biopsy, and in the remaining the type of controls selected is not clear (12, 13) or both healthy subjects and BPH patients were evaluated (16).

All studies measured VEGF levels using ELISA methods. Four studies assessed serum (13, 16, 18, 19) and three assessed plasma VEGF (14, 15, 17), with the latter reporting lower median VEGF levels. Peyromaure et al (18) used an ELISA kit with a polyclonal antibody designed to measure all the five VEGF-A isoforms but in the other studies (13, 14, 16-19) an immunoassay technique designed to measure only the two active isoforms of VEGF-A (VEGF 121 and VEGF 165) was used.

The comparison of VEGF levels between controls and different prostatic pathology groups is presented in figure 2.

Five (12-15, 17) of the eight studies report a statistically significant higher VEGF levels in patients with prostatic adenocarcinoma compared to controls. Three of these reports (14, 15, 17) referred to studies that measured VEGF in the plasma and whose controls had negligible risk of prostatic cancer, although such diagnosis was not excluded by prostatic biopsy. The other two studies evaluated serum VEGF levels (12, 13) and also reported higher levels of VEGF in prostatic cancer patients, but it was not clear the controls' risk of prostatic cancer.

In the two studies (18, 19) that used only controls with risk of prostatic cancer and that measured VEGF in serum, the VEGF levels were higher in patients with benign prostatic disease compared to cancer patients but the results were not statistically significant. Jones et al (16) used controls with and without high risk of prostatic cancer and measured serum VEGF. Controls with negligible risk of cancer had lower VEGF levels and hormone-refractory prostatic cancer patients higher VEGF levels than benign prostatic hyperplasia and hormone-sensitive prostatic cancer, although there were no statistical differences when comparing all the groups.

Three studies (15, 17, 18) showed no statistically significant correlation between VEGF and PSA (one reports a negative and two a positive correlation), but positive ($r=0.35$; $p=0.02$)(16) and negative ($r=-0.72$; $p<0.05$) (12) significant correlations were also reported. No study evaluated the role of VEGF as an indicator of prostatic cancer independently from PSA levels.

Four studies (12, 15, 17, 18) evaluated the relationship between VEGF levels and biopsy Gleason score, with only one (17) showing a significant positive association ($G\leq6$ *vs.* $G\geq7$: 9.6 *vs.* 13.2, $p=0.036$) and two (15, 18) reporting a non-significant positive relation.

Regarding differences in VEGF according to tumor clinical stage, three studies (15, 16, 18) reported higher VEGF levels in metastatic than in localized prostate cancer but the differences were statistically significant only in two (15, 17). Peyromaure et al (18) reported higher levels of VEGF in locally advanced or metastatic tumors ($cT\leq2$: 48 pg/mL; cT3: 66 pg/mL; N+ or M+:104 pg/mL; $p=0.62$), but the results were not statistically significant. Duque et al (15) reports no significant relation between clinical stage and VEGF levels (cT1c: 4.0 pg/mL; cT2: 8.5 pg/mL; cT3: 4.5 pg/mL; $p=0.54$).

Shariat et al (17) have also shown differences according pathological stages. He reposts higher levels of VEGF in patients with adenocarcinoma with extraprostatic extension ($pT\leq2$ *vs.* $pT\geq3$: 9.6 *vs.* 12.4, $p=0.047$) and with lymph node metastasis (pN0 *vs.* pN+: 9.6 *vs.* 29.8, $p<0.001$) in the prostatectomy specimens, but no other study investigated this association. Trapeznikova et al (12) reported no association between VEGF serum levels and stage, but no further information is provided in the abstract.

Discussion

Plasma VEGF levels are higher in prostate cancer patients than in controls with negligible risk of prostate cancer, as confirmed by all the studies that evaluated this association. This difference was not verified when controls with risk of prostatic cancer but negative prostate biopsy were used. Serum VEGF levels were not consistently different across groups of prostate cancer patients with diverse clinical characteristics.

In the present review we systematically evaluated the best available evidence assessing the role of VEGF serum or plasma levels in prostate cancer diagnosis. Our conclusions, however, are limited by the small number of studies identified, despite comprehensive electronic database and cross-reference searches, and by the heterogeneous methodology and presentation of results, in addition to the intrinsic limitations of the primary studies.

Publication bias is a potential source of error in literature reviews, which we tried to overcome in the present study. On the one hand, we conducted a comprehensive search and included in the review two articles written in Russian from which information could be obtained only in the English abstract. On the other hand, VEGF is an investigational marker, not measured by routine, which expectedly increases the probability of publication regardless of the existence of positive findings. Our review includes small studies with non-significant results, which also argues against publication bias.

The articles included in the review used two different ELISA kits, one that measured all five VEGF-A isoforms and the other designed to measure only the two active isoforms of VEGF-A (VEGF 121 and VEGF 165). The results obtained with this kit correlate well with total VEGF, as reported by the manufacturer, and have very good reproducibility (14, 17, 19). Therefore, we do not expect these technical differences to be responsible for the heterogeneous results across studies.

The VEGF concentrations observed were much higher in studies evaluating serum (12, 13, 16, 18, 19) than in those assessing plasma levels (14, 15, 17), which reflects the fact that serum VEGF includes VEGF stored in the platelets' α-granules and released during blood clotting, in addition to plasma VEGF. Cancer patients have a higher platelet load compared to healthy individuals (21) and platelets from patients with breast or prostate cancer contain more VEGF than platelets from age- and sex-matched controls (22). Plasma and serum VEGF levels, however, are strongly correlated and at the moment there is no consensus regarding the more appropriate biological product for assessment of VEGF levels (23-26).

The selection of controls is essential to ensure the validity of the conclusions from studies addressing diagnostic accuracy. Diagnostic tests must be evaluated in clinically relevant populations, preferably in consecutive series of individuals in whom the target condition is suspected (20). Studies using healthy controls, not representing the whole spectrum of potential diagnosis

alternative to prostate cancer, suffer from limited-challenge bias (20, 27) and produce inflated estimates of diagnostic accuracy (28).

The three studies that measured VEGF in plasma were the same that used controls with negligible risk of prostatic cancer, and found statistically higher levels of VEGF in prostatic cancer patients. Conversely, two studies using subjects with some criteria for suspicion of prostate cancer as controls measured VEGF in serum and found no such difference. Although it is not possible to disentangle the effect of both biologic specimen for VEGF measurement and type of controls, one study (16) that evaluated serum levels and controls with and without risk of prostatic cancer found no statistically significant differences between all groups but do report some differences when comparing the extreme groups with all the others (healthy controls and hormone-refractory prostate cancer patients had lower and higher VEGF levels than the remaining participants, respectively). These results support the hypothesis of limited-challenge bias being responsible for biased positive associations between VEGF levels and prostate cancer.

The characteristics of the control group are especially important when assessing the role of VEGF in prostate cancer diagnosis as it is expected to be used as an add-on. Its purpose is not to replace PSA testing or to be used as a screening tool, but to contribute to identify false-positive or false-negative results of the PSA test. Unfortunately, the studies included in this review showing significant differences between prostate cancer patients and controls, used control groups including subjects likely to have low PSA levels that would never been considered for VEGF testing in a clinical setting.

Despite tissue expression of VEGF being associated with tumor Gleason sum in studies using immunohistochemical staining (29) and rapid colorimetric *in situ* hybridization (30), such association was not demonstrated in most of the articles that assessed blood VEGF levels. The results are also inconsistent for the association between total PSA levels and serum or plasma VEGF.

VEGF levels appear to be higher in patients with metastatic prostatic cancer, especially those in hormone-refractory status, compared to localized prostatic cancer, but evidence is inconclusive regarding differences according to clinical local staging.

The conclusion that plasma VEGF levels are higher in patients with prostate cancer compared to healthy controls is unlikely to be of any clinical benefit. PSA level is certainly a better indicator of disease (31) and no study proved that VEGF was an independent predictor of cancer in those with prostate pathology associated with elevated PSA levels.

Serum VEGF levels do not seem clinically useful for selection of patients to be submitted to prostate biopsy. Whether if it is because serum VEGF levels are not as useful as plasma levels, or because patients with benign prostatic pathology but with high risk of prostate cancer have higher VEGF levels than healthy controls remains unanswered by the currently available evidence.

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Figure 1 – Systematic review flowchart.

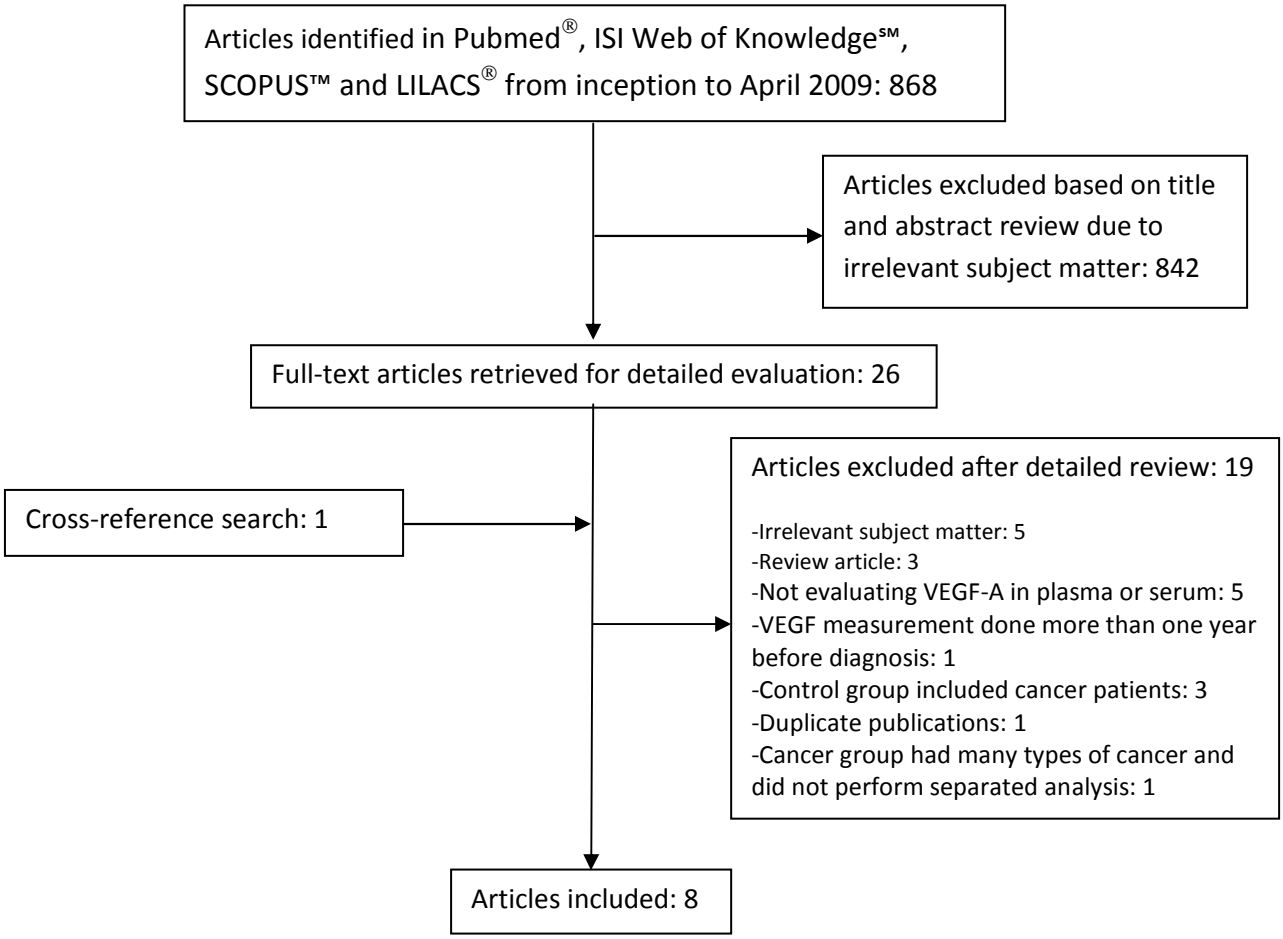
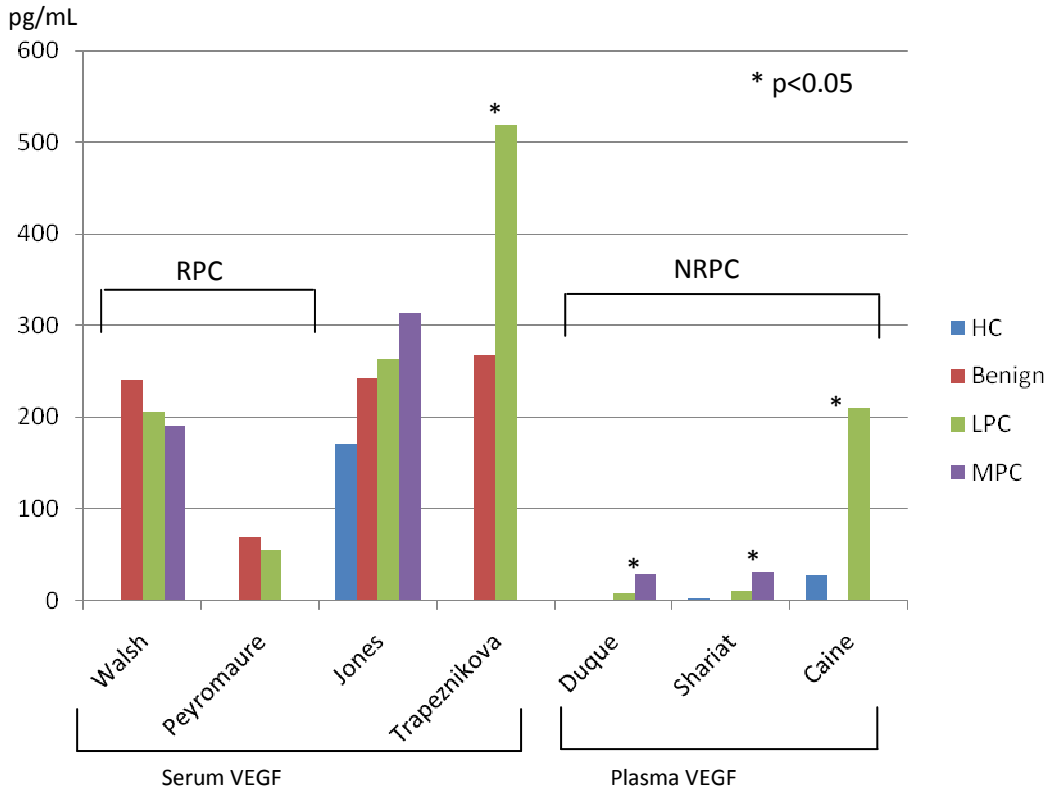


Figure 2 – Vascular Endothelial Growth Factor levels according to prostatic pathology.



RPC: Controls with high risk of prostatic cancer but the prostate biopsy was negative for malignancy; NRPC: Controls with negligible risk of prostate cancer; HC: Healthy controls; Benign: patients with benign prostatic pathology or no prostatic pathology; LPC: localized prostatic cancer, as defined by authors; MPC: metastatic prostatic cancer, as defined by authors;

Notes: one article from Trapeznikova et al (12) included in the systematic review is not presented in this figure as it does not describe VEGF levels in the different prostatic pathologies (just reports that mean VEGF was not significantly higher ($p<0.05$) in LPC than in patients with BPH); The abstract from Trapeznikova et al (13) did not provide information about the type of controls used and Jones et al (16) evaluated healthy controls and controls with BPH whose risk of prostatic cancer is not reported.

Table 1. Summary of the Studies of Vascular Endothelial Growth Factor and Prostatic Cancer Included in the Systematic Review

First author, publication yr Country	Participants	Biological product/method for VEGF measurement	VEGF pg/mL	Notes/Other results
Walsh, 1999 (19) United Kingdon	40 BPH¥ 26 LPC 40MPC	Serum ELISA-1	Median (interquartile range) BPH: 241 (169–324); LPC: 206 (126–954); MPC: 190 (104–285) NS (not further specified)	
Duque, 1999 (15) USA	26 HC 54 LPC 26 MPC	Plasma ELISA-1	Median (interquartile range) HC: 0 (0-24); LPC: 7.0 (0-26.5); MPC: 28.5 (19.3-57.0) p<0.001	- Controls were 17.5 yrs younger, on average - VEGF vs. PSA§: r=0.14 (p=0.22) - VEGF by Gleason¤: ≤6: 5.5; 7: 11.5; ≥8: 23.5 (p=0.18) - VEGF levels stratified by clinical stage in LPC: T1c: 4.0; T2: 8.5; T3: 4.5 (p=0.54)
Jones, 2000 (16) UK	21 HC 9 BPH¥ 16 LPC 32 MPC (including. 9 HRMPC)	Serum ELISA-1	Mean HC: 170.7; BPH: 241.7; LPC: 263.9; MPC: 313.5 HRMPC: 535.0 p>0.05	- Controls were 42 yrs younger, on average - VEGF vs. PSA §: r=0.35 (p=0.02) - VEGF levels in HC and HRMPC were different from the others (p<0.01) but no differences between BPH, LPC or MPC (p>0.05)
Shariat, 2004 (17) USA	40 HC 215 LPC 9 MPC	Plasma ELISA-1	Median (range) HC: 2.24 (1.61-2.99); LPC: 9.91 (1.99-166.9); MPC: 31.30 (15.3-227.1) p<0.001	- VEGF vs. PSA §: r=0.119; p=0.081 - VEGF by Gleason¤: ≤6: 9.6; ≥7: 13.2; p=0.036 - VEGF levels by pathological stage: ≤T2: 9.6; ≥T3: 12.4; (p=0.047); N0: 9.6; N+: 29.8 (p<0.001)

Caine, 2004 (14) UK	30 HC 30 LPC	Plasma ELISA-1	Median (interquartile range) HC: 26.5(25-50); LPC: 210(166-360) p=0.0001	
Trapeznikova, 2004 (13) Russia	80 BPH 38 PC	Serum ELISA-1	Mean BPH: 267.9; PC: 518.9 p<0.001	- VEGF (cut-off: 151.5 pg/ml) Sensitivity, 76.2% Specificity, 57.6%
Peyromaure, 2005 (18) France	20 BPH¥ 27 PC	Serum ELISA-2	Median (interquartile range) Benign: 69.5(34.5-145.5); PC: 55 (25-113) p=0.55	- VEGF vs. PSA §: r=-0.003 (p=0.98) - VEGF by Gleason¤: 6: 47; 3+4: 39; 4+3: 49; ≥8: 159 (p=0.12) - VEGF levels by clinical stage: ≤T2: 48; T3: 66; N+ or M+: 104 (p=0.62)
Trapeznikova, 2005 (12) Russia	36 BPH 25 PC	Serum NSA	mean VEGF was significantly higher (p<0.05) than in patients with BPH, not further specified	- VEGF vs. PSA§: r = 0.72 (p<0.05) in PC patients - No association between VEGF and stage or Gleason score (not further specified)

BPH: benign prostatic hyperplasia; ELISA-1 - ELISA kit (Quantikine, R&D Systems, Minneapolis, MN; also distributed by Abingdon, UK) that measure VEGF-A 121 and 165; ELISA-2 - ELISA kit (Bender MedSystems, Vienna, Austria) that measure all the five VEGF-A isoforms; HC: healthy controls with negligible risk of prostatic cancer; HRMPC: hormone-refractory metastasized prostatic cancer; LPC: localized prostatic cancer; MPC: metastasized prostatic cancer; NS: no significant; NSA: not specified in the abstract; PSA: total prostatic-specific antigen; § Correlation between VEGF levels and total prostatic-specific antigen; ¤VEGF levels stratified by Gleason score in the biopsy; ¥Controls with high risk of Prostatic Cancer based on PSA levels or digital rectal examination but with a negative prostate biopsy

Vascular Endothelial Growth Factor (VEGF) and Prostatic Pathology

Abstract

Background: Previous studies suggest that Vascular Endothelial Growth Factor (VEGF) circulating levels might improve identification of patients with prostate cancer but results are conflicting, probably due limited-challenge bias. We aimed to compare serum VEGF levels across different prostatic pathologies (including benign prostatic hyperplasia, prostatitis, high grade prostate intraepithelial neoplasia and prostate cancer) in patients at high risk of prostate cancer.

Methods: We consecutively enrolled 186 subjects with abnormal digital rectal examination and/or total PSA (tPSA) ≥2.5ng/mL. Blood was collected before diagnostic ultrasound guided trans-rectal prostate biopsy, or any prostate oncology treatment, to measure PSA isoforms and VEGF. Unconditional logistic regression was used to compute age-, tPSA- and free/total PSA-adjusted odds ratios (OR) and respective 95% confidence intervals (95%CI) for the association between serum VEGF and different prostatic pathologies.

Results: Prostate biopsy main diagnoses were: normal or benign prostatic hyperplasia (27.3%), prostatitis (16.6%), and prostatic cancer (55.0%). The median VEGF levels (ng/mL) in these groups were 178.2, 261.3 and 266.4 (p=0.029), respectively, but no significant differences were observed for benign *vs.* malignant pathologies (215.2 *vs.* 266.4, p=0.551). No independent association was observed between VEGF (3rd *vs.* 1st third) and prostatic cancer, when compared to benign conditions (adjusted OR=1.44; IC95%:0.64-3.26).

Conclusions: In patients at high risk of prostatic cancer, circulating VEGF levels have no clinical role in deciding which patients should be submitted to prostatic biopsy. Prostatitis patients, often with higher PSA levels, also present high serum VEGF, and their inclusion in control groups might explain the heterogeneous results in previous studies.

Introduction

Prostate cancer is the most commonly diagnosed non dermatologic malignancy and the third leading cause of cancer mortality among men in Europe (1). Prostatic specific antigen (PSA) is widely used for prostate cancer screening, despite its low accuracy across different cut-offs (2). However, the need to avoid unnecessary biopsies and missed diagnosis has led to the study of several other biomarkers that could further contribute to decide which patients should be referred to prostatic biopsy.

Vascular Endothelial Growth Factor (VEGF) is a growth factor involved in the promotion of endothelial cell proliferation, vascular permeability and angiogenesis, which are critical steps for tumor growth and development, namely prostate cancer (3). It is synthesized by adenocarcinoma cells (4, 5), and in prostatic cancer patients the prostatic gland contributes considerably to circulating VEGF levels (6). Elevated plasma VEGF levels could reflect prostatic VEGF production, making VEGF a potentially interesting tumour marker to support the decision of submitting a patient to prostatic biopsy.

Previous studies on this topic are conflicting. Some authors have found higher levels of VEGF in prostatic cancer patients (7-10), while others found no differences between subjects with benign prostatic hyperplasia (BPH) and those with malignant disease (11, 12), or increased values only in patients with metastatic prostatic cancer (13) or hormone-refractory disease (14). However most previous studies studied relatively small samples (8, 9, 11-14) and all suffered from limited-challenge bias, as prostatitis, which may interfere with the diagnostic value of VEGF, was not evaluated separately in any of the studies and in many studies the control group only included subjects with no suspicion of prostatic cancer (7, 10, 13).

We aimed to evaluate VEGF as a diagnostic tool for prostatic cancer, comparing its serum levels across groups of patients with suspected prostate cancer, presenting different prostatic pathologies (including BPH, prostatitis, high grade prostate intraepithelial neoplasia (HGPIN) and prostate cancer).

Patients and Methods

Patient selection

During 2006 we consecutively enrolled 186 candidates referred to ultrasound guided trans-rectal prostate biopsy, on the basis of abnormal rectal examination and/or elevated total PSA (tPSA) levels (≥ 2.5ng/mL), in the Department of Urology of S. João Hospital. None of the patients received hormonal therapy, radiotherapy or chemotherapy before undergoing prostate biopsy.

Measurement of biomarkers

Blood was collected from all participants prior to biopsy, and samples were allowed to clot for 30 minutes before centrifugation. Part of the serum was used for a new assessment of tPSA, free PSA (fPSA) and complexed PSA (cPSA). The remaining serum was frozen (-20ºC), and later on was used for VEGF quantification by ELISA (quantitative sandwich enzyme immunoassay technique) double determinations with Quantikine®, a Human VEGF Immunoassay (R&D Systems, Minneapolis, MN).

Outcome evaluation

The final prostate pathology and the prostate cancer cases Gleason score were defined by biopsy results. The number of biopsy cores ranged from 8 to 13. All prostatic biopsies were reviewed by two different pathologists that were blinded to the patients' different PSA isoforms and VEGF values. Patients were grouped in four mutually-exclusive groups, according to the most severe diagnosis observed in the biopsy specimens, as follows (ordered by increasing severity): normal prostate or BPH (N/BPH), prostatitis, HGPIN, and prostatic cancer.

Statistical analysis

The Kruskal-Wallis test was used to compare quantitative variables across prostatic pathology groups. Spearman correlation coefficients were computed to quantify the association between VEGF and age, tPSA, cPSA and f/t PSA ratio.

A receiver operating characteristic (ROC) analysis was used to compute the area under the ROC curve (AUC) and to identify the VEGF level cut-off for which a higher proportion of patients was correctly classified when distinguishing prostatic cancer from benign diagnosis.

Unconditional logistic regression was used to compute odds ratios (OR) and respective 95% confidence intervals (95%CI) for the association between serum VEGF levels (groups defined using tertiles as cut-offs and the cut-off defined by the ROC curve analysis) and different prostatic pathologies, crude and adjusted for age, tPSA and f/tPSA. The tPSA levels were modelled after log-transformation. Further analyses were conducted combining N/BPH, prostatitis and HGPIN in a group of benign pathology. Due to the low number of patients with HGPIN, these patients were excluded from the analyses by prostatic pathology subgroups, and considered only when comparing malign with all types of benign pathology.

Statistically significance in this study was set as $p<0.05$. All reported p values are two-sided. Statistical analysis was performed using STATA®, version 9.2.

Results

The median age of the participants was 68 years (percentile 25-percentile 75 [P25-P75]: 62-73), the median tPSA level was 7.4 ng/mL (P25-P75: 5.4-12.1) and the median f/tPSA ratio was 0.16 (P25-P75: 0.08-0.23).

Prostatic biopsies revealed prostatic cancer in 99 cases (53.2%), prostatitis in 30 cases (16.1%), HGPIN in 6 cases (3.2%), BPH in 32 cases (17.2%) and normal prostate in the remaining 19 participants (10.2%). Among prostatic cancer cases the Gleason score was 6 in 21.4% patients, 7 in 51.1% and 8 or higher in 23.4%.

Table 1 summarizes participants' characteristics stratified by prostatic histology. Age was similar between groups, but tPSA and cPSA were significantly higher in patients with prostatitis (9.9 and 6.7 ng/mL, respectively) and prostatic cancer (8.3 and 6.4 ng/mL respectively) when compared to N/BPH (5.7 and 4.0 ng/mL, respectively). The median f/t PSA ratio was lower in prostatic cancer patients compared to patients with benign histology (0.11 vs. 0.21).

The median serum VEGF level in our sample was 232.3 pg/mL (range: 16.4-1648.3 g/mL; P25-P75: 144.0-339.6 pg/mL). There was a weak positive correlation between VEGF and tPSA (r=0.18; p=0.013) and a weak negative correlation between VEGF and f/t PSA ratio (r=-0.17; p=0.017). No significant association was observed with age (r=-0.04; p=0.56) or cPSA (r=0.15; p=0.054). These results were similar when stratified by prostatic pathology (data not shown).

As presented in Figure 1, VEGF levels were significantly higher in prostatic cancer and prostatitis than in N/BPH (median: 266.4, 261.3 and 178.2 pg/mL, respectively; p=0.029), but no statistically significant difference was observed when comparing prostatic cancer with benign pathology (median: 215.2 *vs.* 266.4 pg/mL, respectively; p=0.551). The median VEGF levels were similar when the analysis was restricted to patients with tPSA between 2.5 and 10 ng/mL (211.5 pg/mL for benign histology and 246.7 pg/mL for prostatic cancer; p=0.67). These results were similar if patients with HGPIN were excluded (data not shown).

The ROC curve of VEGF serum levels for the detection of prostatic malignancy is presented in figure 2. The AUC was 0.53 (95%CI: 0.44-0.61) and the cut-off value for which a higher proportion of patients was correctly classified (57.0%) was 266.4 pg/mL.

Higher VEGF levels (3rd third *vs.* 1st third) were approximately twice more likely in patients with prostate cancer compared to N/BPH, but the adjusted estimates were not significantly different from unity (OR=2.19, 95%CI: 0.76-6.31) (table 2).

Results comparing benign and malignant prostatic pathology are presented in table 3. In general, OR estimates were lower than when N/BPH was used as reference. The age- and PSA-adjusted OR for the association between prostatic cancer and higher VEGF levels (3rd third *vs.* 1st third) was 1.44 (95%CI: 0.64-3.26).

In prostatic cancer patients the VEGF levels were not significantly different across Gleason score groups. The median values were 258.8 pg/mL for patients with histological Gleason score 6, were 272.5 pg/mL for those with Gleason score 7 and 234.8 pg/mL for those with the more aggressive score Gleason score 8-10 (p=0.716).

Discussion

VEGF levels are higher in subjects with prostatitis and prostatic cancer compared to patients at high prostate cancer risk but whose prostatic biopsy only revealed normal or hyperplasic tissue. However, in this consecutive series of patients eligible for prostatic biopsy there were no overall differences in VEGF serum levels between subjects with benign prostatic disease and prostate cancer cases.

Our results contribute to explain the heterogeneity observed in the literature on this topic. Prostatitis is an inflammatory condition associated with angiogenesis that raises VEGF levels, similar to the observed in prostate cancer, and may be highly prevalent in patients with increased tPSA levels. Reports of prostatitis prevalence range from 10% to 63% (15), and it was 16.1% in our series. We observed no relevant difference in VEGF circulating levels between patients with benign prostatic histology and cancer, when patients with prostatitis were also considered in the latter group. The two previous studies (11, 12) that evaluated participants with high risk of prostate cancer also observed no significant associations between cancer and VEGF levels.

Other studies (7-10, 13, 14) showed higher VEGF levels in patients with prostate cancer when compared with healthy controls or subjects with benign prostatic hypertrophy. Such comparisons however, are not clinically relevant since elevated tPSA is the most frequent indication for prostatic biopsy, and reflect limited-challenge-bias (16, 17). A diagnostic test must be evaluated in a clinically relevant population, preferably in a consecutive series of individuals in whom the target condition is suspected (17). Studies using healthy controls, not representing the whole spectrum of potential diagnosis alternative to prostate cancer which are able to generate false-positive results, namely when prostatitis is present, produce inflated estimates of diagnostic accuracy (18).

Also, in some of these studies (7, 10, 13, 14) whose controls were not suspected of having prostate cancer, the investigators did not perform any biopsy in the individuals that were categorized as healthy or only presenting BPH based on low PSA levels and a negative digital rectal examination. However, Thompson et al (19) detected prostatic cancer in 10.1 percent among those with values of 0.6 to 1.0 ng/mL, 17.0 percent among those with values of 1.1 to 2.0 ng/mL, 23.9 percent among those with values of 2.1 to 3.0 n/mL, and 26.9 percent among those with values of 3.1 to 4.0 ng/mL. These can lead to a differential information bias that would cause an underestimation of the true association measure.

VEGF could also be important in clinical practice if its levels were higher in patients with worst prognosis prostatic cancer (those with higher Gleason score or in higher clinical stage). In

our study we did not find significant associations between VEGF levels and Gleason score in the 99 prostate cancer patients, in accordance with previous reports (8, 11, 20, 21). Only Shariat et al (7) describe higher VEGF levels in those with higher Gleason score, and Duque et al (13) report higher levels in patients with Gleason score ≥8 although no positive relation between Gleason score and plasma VEGF was observed. Differences in VEGF levels between metastatic and localized prostatic cancer have been reported (13), but we decided not to make such evaluation in our study due to the restricted number of cases.

To measure serum VEGF we used the kit from R&D Systems that has an intra-assay coefficient of variation of 4.5% (22). This Elisa kit has been used previously (7, 12, 13) and is considered adequate to measure VEGF in serum or plasma.

Circulating VEGF in serum from cancer patients may reflect an aggregate of tumor-cell and platelet-stored VEGF (23). To better reflect the disease-related circulating VEGF levels, the use of rapidly processing citrated plasma samples and additional centrifugation has been recommended (23). This has been disputed, with other authors suggesting that both plasma and serum levels of VEGF may be equally useful (24). Nonetheless there is a potential for an information bias in our VEGF levels that we cannot exclude, although its effects are difficult to predict.

The use of circulating VEGF to predict disease staging, patient outcome, early identifying patients at higher risk of lymph node metastases or selecting patients for early systemic intervention or adjuvant radiation therapy, sparing others from the associated morbidity with these treatment options, are still under study and can provide important advances in prostate oncology. Ultimately, a better understanding of the VEGF system should provide additional knowledge about prostatic cancer growth that would allow us to develop better molecular markers to use in clinical practice.

Our results show that VEGF levels have no clinical importance in deciding which patients suspected of having prostatic cancer should be submitted to prostatic biopsy. The exclusion of patients with prostatitis from the control group is the probable cause of the heterogeneous results in previous studies.

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Table 1. Characteristics of the participants stratified by prostatic histology.

		N/BPH	Prostatitis	HGPIN	Prostate Cancer	p
n		51	30	6	99	
median Age (years)		67.0	67.0	67.0	69.0	0.678
Age	**≤60**	13 (25.5%)	5 (16.7%)	1 (16.7%)	16 (16.2%)	0.766
(years)	**60-70**	20 (39.2%)	15 (50.0%)	3 (50.0%)	40 (40.4%)	
	>70	18 (35.3%)	10 (33.3%)	2 (33.3%)	43 (43.4%)	
median tPSA (ng/mL)		5.7	9.9	6.1	8.3	<0.001
tPSA	**≤4**	11 (22.0%)	1 (3.4%)	1 (16.7%)	10 (10.1%)	0.006
(ng/mL)	**4-10**	33 (66.0%)	14 (48.3%)	4 (66.7%)	51 (51.5%)	
	>10	6 (12.0%)	14 (48.3%)	1 (16.7%)	38 (38.4%)	
median cPSA (ng/mL)		4.0	6.7	4.6	6.4	<0.001
cPSA	**≤4**	26 (51.0%)	5 (1.2%)	3 (50.0%)	18 (18.6%)	<0.001
(ng/mL)	**4-10**	24 (47.1%)	21 (72.4%)	3 (50.0%)	53 (54.6%)	
	>10	1 (2.0%)	3 (10.3%)	0 (0.0%)	26 (26.8%)	
median f/tPSA ratio		0.22	0.20	0.22	0.11	<0.001
f/tPSA ratio	**≤0.15**	14 (28.0%)	10 (34.5%)	0 (0.0%)	68 (68.7%)	<0.001
	>0.15	36 (72.0%)	19 (65.5%)	6 (100.0%)	31 (31.3%)	
median VEGF (pg/mL)		178.2	261.3	251.9	266.4	0.067
VEGF *	**≤170**	24 (47.1%)	6 (20.0%)	2 (33.3%)	31 (31.3%)	0.087
(pg/mL)	**171-335**	18 (35.3%)	11 (36.7%)	3 (50.0%)	31 (31.3%)	
	>335	9 (17.6%)	14 (43.3%)	1 (16.7%)	37 (37.4%)	

* Tertiles were used to define cut-offs.

N/BPH: normal prostate or benign prostate hyperplasia; HGPIN: high grade prostate intraepithelial neoplasia;

Table 2. Multivariate logistic analysis of the association between vascular endothelial growth factor and prostatic histology.

	N/BPH	Prostatitis			Prostate Cancer		
Serum VEGF (pg/mL)	n (%)	n (%)	OR (95% CI)	OR* (95% CI)	n (%)	OR (95% CI)	OR* (95% CI)
<266.4§	37 (72.6)	16 (53.3)	1 (reference)	1 (reference)	50 (50.5)	1 (reference)	1 (reference)
>266.4§	14 (27.4)	14 (46.7)	2.31 (0.90-5.95)	1.63 (0.53-5.03)	49 (49.5)	2.59 (1.25-5.38)	1.89 (0.81-4.38)
≤170†	24 (47.1)	6 (19.4)	1 (reference)	1 (reference)	31 (31.3)	1 (reference)	1 (reference)
171-335†	18 (35.3)	11 (35.5)	5.78 (1.68-19.85)	4.57 (1.00-20.94)	31 (31.3)	3.18 (1.29-7.85)	2.18 (0.79-6.05)
>335†	9 (17.6)	14 (45.2)	2.36 (0.76-7.34)	1.05 (0.28-3.99)	37 (37.4)	2.39 (0.94-6.06)	2.19 (0.76-6.31)

* adjusted for age, tPSA and f/tPSA;

§ - cut-off that optimizes proportion of patients correctly classified

† - tertiles were used to define cut-offs

N/BPH: normal prostate or benign prostate hyperplasia

Table 3. Multivariate Logistic Analysis of the association of Vascular Endothelial Growth Factor with prostate cancer.

	Benign Histology	Prostate Cancer		
Serum VEGF (pg/mL)	n (%)	n (%)	OR (95% CI)	OR* (95% CI)
<266.4§	56 (64.4)	50 (50.5)	1 (reference)	1 (reference)
>266.4§	31 (35.6)	49 (49.5)	1.77 (0.98-3.19)	1.22 (0.62-2.40)
≤170†	32 (36.8)	31 (31.3)	1 (reference)	1 (reference)
171-335†	32 (36.8)	31 (31.3)	1.66 (0.81-3.40)	1.01 (0.44-2.31)
>335†	23 (26.4)	37 (37.4)	1.66 (0.81-3.40)	1.44 (0.64-3.26)

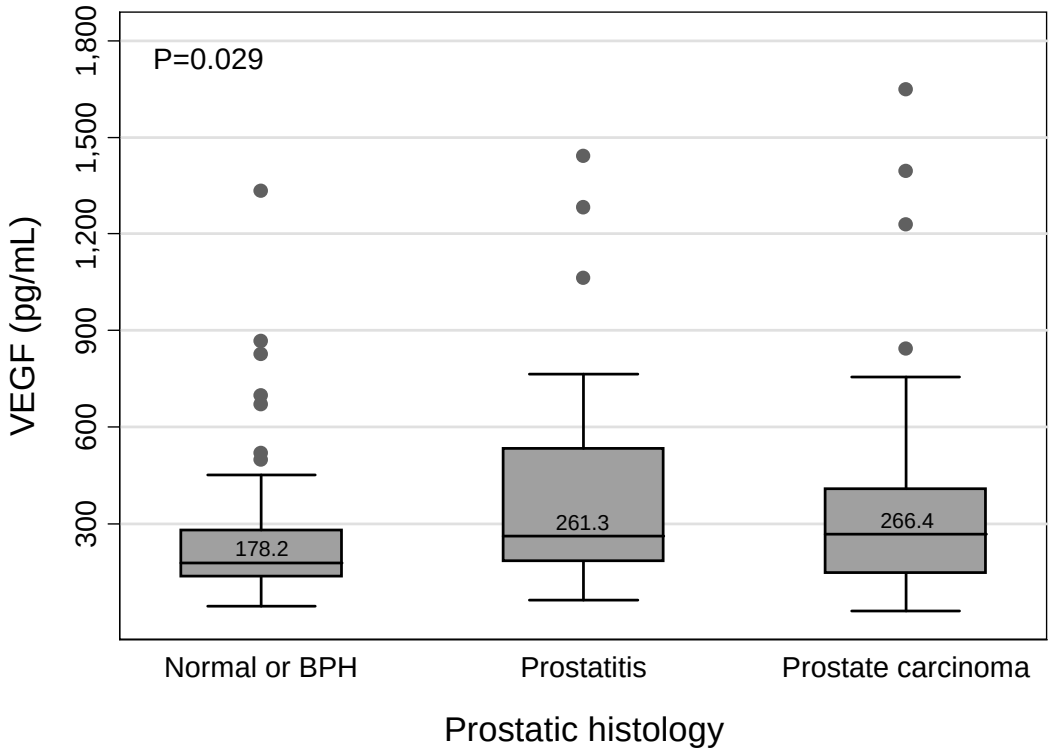
* adjusted for age, tPSA and f/tPSA

§ - cut-off that optimizes proportion of patients correctly classified

† - tertiles were used to define cut-offs

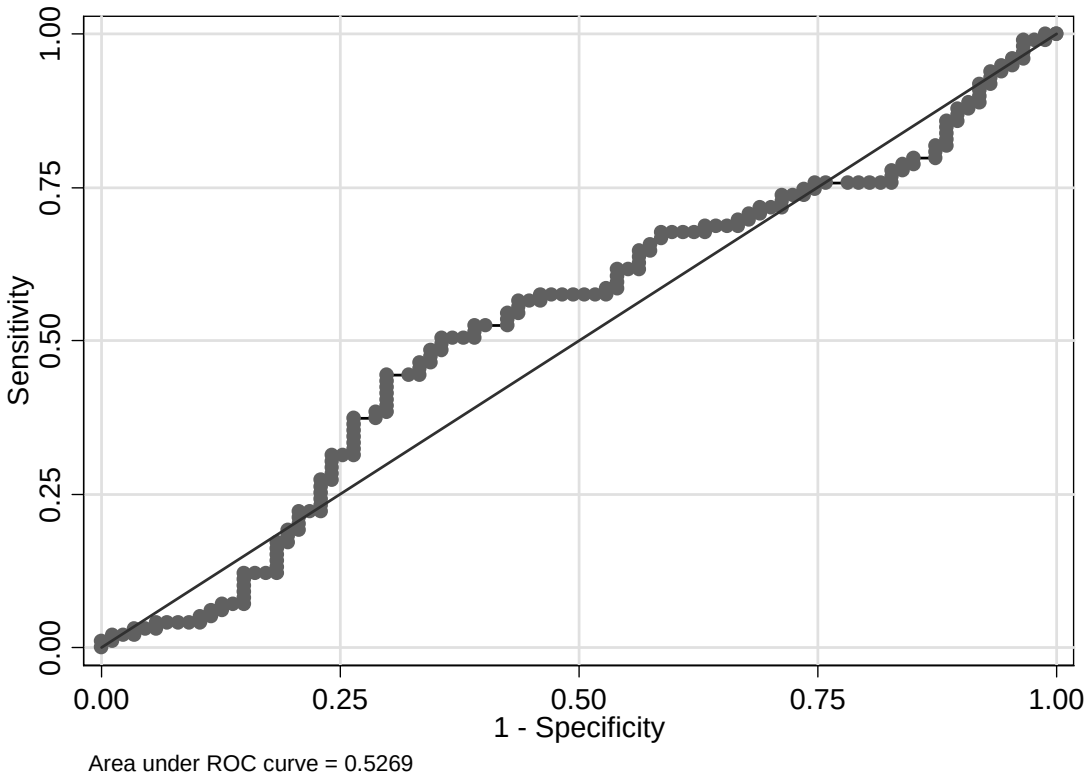
Benign Histology – including normal prostate or benign prostate hyperplasia, prostatitis and HGPIN

Figure 1. Serum levels of Vascular Endothelial Growth Factor (VEGF), according to prostatic biopsy histology.



BPH: Benign Prostatic Hyperplasia

Figure 2. Receiver operating characteristic (ROC) curves for VEGF serum levels as a test for diagnosis prostatic carcinoma using the biopsy results as the gold standard.



7. Summary and Conclusions

Prostate cancer is the second most frequent malignant neoplasm and the sixth cause of cancer death in the world, with about half a million cases and two hundred thousand deaths in 2002. It is often suspected during a routine check-up when an elevated serum level of PSA (Prostate Specific Antigen) in the blood test is detected, and the diagnosis is usually achieved by prostate biopsy under ultrasound guidance. However, PSA lacks both sensitivity and specificity and its use as a screening tool remains controversial. The need to avoid unnecessary biopsies and missed diagnosis has lead to the study of several other biomarkers that could further contribute to decide which subjects should be referred to prostatic biopsy.

VEGF is a growth factor involved in the promotion of endothelial cell proliferation, vascular permeability and angiogenesis, which are critical steps for tumor growth and development, namely prostate cancer. It is synthesized by prostatic adenocarcinoma cells, and in prostate cancer patients the prostatic gland contributes considerably to circulating VEGF levels. This makes VEGF a potentially interesting tumour marker to detect patients with high prostate cancer risk.

Two investigations were conducted, with the following specific objectives:

- To review systematically the published studies assessing the role of VEGF serum or plasma levels in prostate cancer detection (manuscript 1).

- To evaluate VEGF as a biomarker for early detection of prostate cancer, comparing its serum levels across groups of patients with suspected prostate cancer, presenting different prostatic pathologies, including benign prostatic hyperplasia, prostatitis, high grade prostate intraepithelial neoplasia (HGPIN) and prostate cancer (manuscript 2).

Manuscript 1 - VEGF and prostatic cancer: a systematic review

Published studies addressing the relation between serum or plasma VEGF levels and prostate cancer were identified searching Pubmed®, ISI Web of Knowledge℠, SCOPUS™ and LILACS® up to April 2009, and reviewed following a standardized protocol.

Three studies reported higher plasma VEGF (pg/mL) in patients with localized prostate cancer than in healthy controls (7.0 *vs.* 0.0, 9.9 *vs.* 2.2, and 210 *vs.* 26.5, $p<0.01$), and two showed higher serum VEGF (pg/mL) in prostate cancer patients than in patients with benign prostate hypertrophy (518.9 *vs.* 267.9, $p<0.001$; no specific values, $p<0.05$). In one study, serum VEGF was significantly lower in healthy controls than in patients with benign prostate hypertrophy, localized or metastatic prostate cancer.

The only two studies that used controls with previous suspicion of prostatic cancer but a negative biopsy reported non-statistically significant difference in VEGF serum levels (pg/mL) between controls and localized prostate cancer patients (206 *vs.* 241 and 69.5 *vs.* 55).

Manuscript 2 - Vascular Endothelial Growth Factor (VEGF) and Prostatic Pathology

We consecutively enrolled 186 subjects with abnormal digital rectal examination and/or total PSA (tPSA) ≥2.5ng/mL. Blood was collected before ultrasound guided trans-rectal prostate biopsy, or any prostate oncology treatment, to measure PSA isoforms and VEGF. Unconditional logistic regression was used to compute age-, tPSA- and free/total PSA-adjusted odds ratios (OR) and respective 95% confidence intervals (95%CI) for the association between serum VEGF and different prostatic pathologies.

Prostate biopsy main diagnoses were: normal or benign prostatic hyperplasia (27.3%), prostatitis (16.6%), and prostatic cancer (55.0%). The median VEGF levels (ng/mL) in these groups were 178.2, 261.3 and 266.4 ($p=0.029$), respectively, but no significant differences were observed for benign *vs.* malignant pathologies (215.2 *vs.* 266.4, $p=0.551$). No independent association was observed between VEGF (3^{rd} *vs.* 1^{st} third) and prostatic cancer, when compared to benign conditions (adjusted OR=1.44; IC95%:0.64-3.26).

Conclusions

- Higher VEGF plasma levels are observed in prostatic cancer patients compared to healthy controls, but serum levels do not appear useful in differentiating

benign from malignant prostatic disease when using, as controls, individuals with high risk of prostate cancer and negative biopsy.

- In patients at high risk of prostatic cancer, serum VEGF levels have no clinical role in deciding which subjects should be submitted to prostatic biopsy. Prostatitis patients, often with higher PSA levels, also present high serum VEGF, and their inclusion in control groups might explain the heterogeneous results in previous studies.

8. Sumário e Conclusões

O cancro da próstata é a segunda neoplasia maligna mais frequente no mundo e a sexta causa de morte por doença neoplásica, com cerca de meio milhão de casos e duzentas mil mortes em 2002. Geralmente é detectado através de análises de rotina, quando o doseamento do PSA (*Prostate Specific Antigen*) se encontra elevado, sendo o diagnóstico confirmado geralmente através de biopsia prostática guiada por ecografia. Contudo, o PSA tem baixa sensibilidade e especificidade e o seu interesse com método de rastreio permanece controverso. Com o intuito de evitar biópsias desnecessárias e falsos negativos, estudaram-se outros biomarcadores que poderão contribuir para decidir quais os indivíduos que deverão ser referenciados para biópsia.

O VEGF é um factor de crescimento envolvido na promoção da proliferação das células endoteliais, permeabilidade vascular e angiogénese, etapas críticas na carcinogénese tumoral, nomeadamente no cancro da próstata. É sintetizado pelas células do adenocarcinoma prostático e, nos doentes com cancro da próstata, a glândula prostática contribui também de forma relevante para os seus níveis séricos. Estes dados tornam o VEGF num biomarcador com potencial para detectar doentes com risco elevado de cancro da próstata.

Foram realizadas duas investigações com os seguintes objectivos específicos:

- Rever sistematicamente os estudos publicados que avaliaram o papel do VEGF sérico ou no plasma na detecção de cancro da próstata (manuscrito 1).

- Avaliar o VEGF como um biomarcador para a detecção precoce do cancro da próstata, comparando os seus níveis séricos em diferentes grupos com essa suspeita, incluindo hiperplasia benigna da próstata, prostatite, neoplasia intra-epitelial de alto grau e cancro da próstata (manuscrito 2).

Manuscrito 1 - VEGF and prostatic cancer: a systematic review

Foram identificados através de pesquisas nas bases Pubmed®, ISI Web of Knowledge℠, SCOPUS™ e LILACS® artigos publicados até Abril de 2009 que avaliaram a relação entre o valor sérico ou plasmático do VEGF. Os estudos identificados foram revistos seguindo um protocolo estandardizado.

Três artigos relataram valores mais elevados de VEGF plasmático (pg/mL) nos doentes com carcinoma da próstata localizado em comparação com controlos saudáveis (7,0 *vs.* 0,0; 9,9 *vs.* 2,2; 210 *vs.* 26,5; p<0,01) e dois demonstraram valores de VEGF sérico (pg/mL) superiores em doentes com cancro da próstata comparados com doentes com hiperplasia benigna da próstata (518,9 *vs.* 267,9, p<0,001; sem valores especificados no artigo, p<0,05). Num estudo, o valor sérico de VEGF foi significativamente inferior em controlos saudáveis do que em doentes com hipertrofia benigna da próstata e cancro da próstata localizado e metastático.

Os únicos estudos que usaram controlos com suspeita prévia de cancro da próstata com biopsia negativa reportaram diferenças estatisticamente não significativas nos níveis séricos de VEGF (pg/mL) entre controlos e doentes com carcinoma localizado da próstata (206 *vs.* 241; 69,5 *vs.* 55).

Manuscrito 2 - Vascular Endothelial Growth Factor (VEGF) and Prostatic Pathology

Foram incluídos neste estudo 186 doentes com toque rectal suspeito e/ou PSA total (PSAt) ≥2,5ng/mL seleccionados consecutivamente. Foi colhida uma amostra sanguínea, colhida antes da biopsia prostática guiada por ecografia ou qualquer tratamento oncológico, para medir as isoformas do PSA e o VEGF. Foram calculados odds ratios ajustados para a idade, PSAt e razão PSA livre/total, e respectivos intervalos de confiança a 95% (IC95%) para quantificar a associação entre os níveis de VEGF sérico e as diferentes patologias prostáticas, utilizada regressão logística não condicional.

Os principais diagnósticos da biopsia prostática foram: normal ou hiperplasia benigna da próstata (27,3%), prostatite (16,6%) e cancro da próstata (55,0%). As medianas dos valores de VEGF (ng/mL) nestes grupos foram 178,2, 261,3 e 266,4 (p=0,029), respectivamente, mas não foram detectadas diferenças estatisticamente significativas entre patologia benigna e maligna (215,2 *vs.* 266,4; p=0,551). Não foi observada associação independente entre VEGF (1º e 3º terço) e cancro da próstata, comparada com patologias benignas (OR ajustado=1.44; IC95%: 0.64-3.26).

Conclusões

- Foram observados níveis mais elevados de VEGF nos doentes com cancro da próstata comparados com os controlos saudáveis, mas os níveis séricos não aparentam ter utilidade na diferenciação entre patologias benignas e malignas, se forem usados controlos com suspeita de cancro da próstata mas biopsia negativa para cancro.

- Nos doentes com suspeita de cancro da próstata, os níveis séricos de VEGF não têm utilidade clínica na decisão sobre que doentes deverão ser submetidos a biópsia prostática. Os doentes com prostatite, frequentemente portadores de níveis elevados de PSA, também apresentam níveis elevados de VEGF, e a sua inclusão nos grupos controlo permite explicar a heterogeneidade dos resultados obtidos em estudos anteriores.