USE OF STATINS AND SERUM LEVELS OF PROSTATE SPECIFIC ANTIGEN

Dissertação de candidatura ao grau de Mestre em Saúde Pública, apresentada à Faculdade de Medicina da Universidade do Porto, realizada sobre orientação do Professor Doutor Nuno Lunet e coorientação do Dr. Francisco Pina

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Ao abrigo do Art.º 8º do Decreto-Lei nº 288/70 esta dissertação tem como base um manuscrito, no qual colaborei ativamente na operacionalização das hipóteses, recolha, análise e interpretação dos dados e fui responsável pela redação da sua primeira versão:

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Aos meus pais, de quem e para quem tudo sou.
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<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>5-ARI</td>
<td>5-α-reductase inhibitors</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BPH</td>
<td>Benign Prostate Hyperplasia</td>
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<tr>
<td>DALY</td>
<td>Disability-adjusted Life Years</td>
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<tr>
<td>DDD</td>
<td>Defined Daily Dosage</td>
</tr>
<tr>
<td>DRE</td>
<td>Digital Rectal Examination</td>
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<tr>
<td>ERSPC</td>
<td>European Randomised Study of Screening for Prostate Cancer</td>
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<tr>
<td>FPP</td>
<td>Farnesyl-pyrophosphate</td>
</tr>
<tr>
<td>GGPP</td>
<td>Geranyl-geranyl-pyrophosphate</td>
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<tr>
<td>HDI</td>
<td>Human Development Index</td>
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<tr>
<td>HMG-CoA</td>
<td>Hydroxy-Methylglutaryl-Coenzyme A</td>
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<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>OECD</td>
<td>Organization for Economic Co-operation and Development</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PLCO</td>
<td>Prostate Lung Colorectal and Ovarian screening trial</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate Specific Antigen</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Control Trials</td>
</tr>
<tr>
<td>RR</td>
<td>Risk Ratio</td>
</tr>
<tr>
<td>TRUS</td>
<td>Trans-rectal Ultrasonography</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>YLD</td>
<td>Years Lived with Disability</td>
</tr>
<tr>
<td>YLL</td>
<td>Years of Life Lost due to premature mortality</td>
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1. RESUMO

Introdução: O cancro da próstata é um dos principais responsáveis pela elevada carga associada ao cancro, em todo o mundo. O uso generalizado do antigénio específico da próstata (PSA), como ferramenta de rastreio, pode ajudar a explicar o aumento observado nas taxas de incidência de cancro da próstata, mas o seu papel na diminuição das taxas de mortalidade continua controverso. O diagnóstico definitivo de cancro depende da confirmação histopatológica após biópsia, pelo que, antes de referir um doente para a realização deste procedimento invasivo, o médico deverá ter em conta os valores séricos de PSA, de acordo com a idade do doente, o seu índice de massa corporal e a medicação habitualmente usada, nomeadamente, estatinas. As estatinas são os agentes antidislipidemicos mais prescritos em todo o mundo e apresentam, para além das propriedades antidislipidemicas, propriedades anti-inflamatórias, anti-invasivas, inibidoras do crescimento tumoral e da angiogénese, potencialmente reduzindo o risco de cancro da próstata. As estatinas também podem contribuir para um decréscimo nos níveis de PSA, através da diminuição dos níveis de colesterol – uma vez que este é um percursor necessário para a síntese de PSA – bem como através da ação sobre o tecido prostático benigno. O impacto das estatinas nos níveis de PSA poderá levar a uma diminuição e um atraso na deteção de cancro quando se usam os valores de referência habituais, pelo que poderá ser necessário ajustar esses valores de referência, de maneira a maximizar a sensibilidade e a especificidade do PSA entre utilizadores de estatinas.

Objetivos: Quantificar o efeito das estatinas nos valores de PSA em doentes referidos para biópsia prostática e determinar se o uso de estatinas deve ser tido em conta para melhorar a precisão do PSA, enquanto ferramenta de diagnóstico de cancro da próstata.

Métodos: Foram selecionados 551 doentes com PSA ≤ 10,0ng/mL, referidos para biópsia prostática por apresentarem PSA ≥ 2,5ng/mL ou um achado anormal no exame de toque retal. Os resultados patológicos foram definidos pela biópsia e as informações relativamente ao uso de estatinas foram recolhidas dos registos clínicos dos doentes. Usámos a ferramenta matemática “path analysis” para quantificar o efeito das estatinas no PSA, tendo em conta variáveis escolhidas de entre aquelas que se associam a uma relação causal entre as estatinas e o PSA. Também usámos Receiver Operating Characteristic curves para avaliar a precisão de modelos, que incluíam como variáveis: os valores de PSA, a idade, o índice de massa corporal, o uso de inibidores da 5-α-reductase, o uso de aspirina e estatinas, para distinguir entre cancro da próstata ou situações benignas.

Resultados: Observou-se um efeito total das estatinas sobre os níveis de PSA (β=-0.283; IC 95%:-0.618, 0.051), principalmente devido ao efeito direto (estatinas→PSA) (β=-0.243; IC 95 %:-0.587,0.101), em vez do efeito indireto (estatinas→cancro→PSA) (β=-0.040; IC 95%:-
Não houve diferenças estatisticamente significativas entre as áreas sob a curva correspondentes aos modelos com ou sem estatinas ($P=0.554$). Este estudo é limitado pela sua natureza transversal, pela falta de informação sobre a duração do tratamento com estatinas, e também pelo facto de os resultados não poderem ser extrapolados para populações um risco diferente de cancro da próstata.

**Conclusão:** Numa amostra consecutiva de pacientes referidos para biópsia prostática, o uso habitual de estatinas foi associado a níveis mais baixos de PSA, principalmente devido a uma ação direta sobre a síntese e metabolismo do PSA, embora com pouco impacto sobre o valor preditivo do teste de PSA nesta população específica.
2. ABSTRACT

**Background**: Prostate cancer is one of the major contributors to the high burden of cancer worldwide. The widespread of Prostate Specific Antigen (PSA) as a screening tool might explain the increase observed in incidence rates but the role of PSA in the mortality decrease is a matter of discussion. The diagnosis of prostate cancer relies on the histopathologic confirmation of cancer after biopsy, but to refer for undergoing this procedure, physicians must take into account serum PSA levels, according to patients’ age, body mass index and medication used, namely statins. Statins are the most prescribed anti-dyslipidemic drugs worldwide and present anti-inflammatory, anti-invasive, tumour growth suppressing, apoptotic and angiogenesis inhibiting properties – in addition to the lipid lowering effects – potentially decreasing the risk of prostate cancer. Statins also contribute to a reduction in total PSA levels, reflecting the lowering of cholesterol levels – a precursor in the synthesis of PSA, as well as due to their action on benign prostatic tissue. The impact of statins on serum PSA levels may lead to a decrease and a delay in prostate cancer detection when using the traditional cut-offs for reference to biopsy, and adjustment of the PSA threshold to maximize sensitivity and specificity may be needed among statins’ users.

**Objectives**: To quantify the effect of statins’ use on Prostate Specific Antigen levels in patients referred to prostate biopsy and to determine if the exposure to statins must be considered to improve the prostate cancer diagnostic accuracy of PSA as screening tool.

**Methods**: We selected 551 patients with PSA ≤10.0 ng/mL referred to ultrasound guided trans-rectal prostate biopsy due to PSA ≥2.5 ng/mL or abnormal digital rectal examination. Results of the prostatic pathology assessment (cancer versus non-cancer) were defined by biopsy and information regarding statins’ use was obtained from patients’ clinical records. We used path analysis to quantify the effect of statins’ use on PSA, taking into account variables chosen to assume a causal relation between statins and PSA levels and the potential confounders. We also used Receiver Operating Characteristic curves to assess the global predictive accuracy of models including PSA, age, body mass index, 5-α-reductase inhibitors, aspirin and statins’ use for distinguishing between prostate cancer and benign conditions.

**Results**: We observed a negative total effect of statins on PSA levels (β=-0.283; 95% CI: -0.618, 0.051), mostly due to the direct effect (statins → PSA) (β=-0.243; 95% CI: -0.587, 0.101) rather than the indirect effect (statins → cancer → PSA) (β=-0.040; 95% CI: -0.178, 0.097). There were no statistically significant differences between the area under the curve corresponding to the models with or without statins (P=0.554). This study is limited by its cross-sectional nature, lack of information on the duration of treatment with statins and also
by the fact that results cannot be extrapolated to settings with a different risk of prostate cancer.

**Conclusions:** In a consecutive sample of patients referred to prostate biopsy, the current use of statins was associated with lower PSA levels, mainly due to a direct action on PSA synthesis and metabolism, though this had little impact on the predictive value of PSA testing in this setting.
3. INTRODUCTION

3.1. Trends in prostate cancer incidence, survival and mortality

Worldwide, prostate cancer is the second most frequent cancer and the sixth cause of oncological death in men, with 899,000 incident cases and 258,000 deaths estimated to have occurred in 2008 (1). There are major differences across countries regarding prostate cancer incidence, with the highest rates observed in the most developed settings and the lowest in some countries from South-Central Asia (1).

Recent analyses of incidence trends show that the rates increased steeply since the late 1980s and stabilized in recent years in developed countries (2-4). The upward trends are mainly due to the introduction and widespread of Prostate Specific Antigen (PSA) testing as a screening tool, and resulted in an increase in the diagnosis of localized cancers and a decrease in the diagnosis of severe, metastatic disease (2-4).

Associated with the use of PSA testing there was an increase in overdiagnosis and in adverse outcomes such as erectile dysfunction and urinary incontinence which contributes to the high morbidity associated with the diagnosis of prostate cancer (5, 6). To measure the burden of this disease, the quantification of disability-adjusted life-years (DALYs) allows for the assessment of the degree of illness and disability in patients and long-term survivors (7). DALYs are composed by the years of life lost because of premature death (YLLs) and the years lived with disability (YLDs) (7). Globally, in 2008, the YLLs were the main contributors for the DALYs attributable to cancer; however, the relative contribution varied by region: on countries with high human development index (HDI), YLLs were 52% of the DALYs for prostate cancer, while in countries with low HDI the contribution from YLLs was 96% (6). There was a high contribution from the YLDs due to cancer in the Americas, most European countries, Australia and New Zealand and most of the sub-Saharan Africa (6).

Despite the high burden, prostate cancer presents high survival rates for which the early detection and early treatment have contributed (8, 9). In the United States of America (USA), the 5-year standardized relative survival was 91.1% among the cases diagnosed between 1990-94 and followed-up to 1999, which is the highest in developed countries (8). In Europe, the overall prostate cancer 5-year standardized survival was 77% in the period 1995-1999, with high variability across countries, ranging from 84.9% in Austria and 47.7% in Denmark (9).

Although the effectiveness of PSA testing and radical treatment remain open to discussion (10, 11) they are usually indicated as the reasons for the decrease observed in mortality rates in several high income countries (12, 13). However, there are some countries,
mainly from Southern and Eastern Europe, that despite the generally low mortality rates, show an increase in mortality rates through the last decades (14).

Regarding treatment, surgery has an arguable efficacy (11) but it has been well demonstrated that radiotherapy as a primary treatment improves overall survival (15), immediate postoperative radiotherapy improves 5-year biochemical progression-free survival (16) and hormone therapy is associated with a longer time to disease progression in metastatic disease and longer overall survival when associated with radiotherapy in locally advanced disease (17).
3.2. Prostate cancer screening and diagnosis

Screening aims to detect disease in asymptomatic people in the earliest stage possible and to allow for a more effective treatment, ultimately leading to a decrease in overall and disease specific mortality (18). Screening for prostate cancer is performed using serum PSA and Digital Rectal Examination (DRE) (19).

Despite the PSA testing has been widely used for early diagnosis, its use as a screening tool at a population level is not supported by the available evidence (18). The most recent review of the studies regarding prostate cancer screening (18) identified five relevant randomised controlled trials, namely the European randomised study of screening for prostate cancer (ERSPC) (20) and the Prostate Lung Colorectal and Ovarian screening trial (PLCO) (21), as the ones with low risk of bias, and the Norrkoping (22), the Quebec (23) and the Stockholm (24) studies as the ones with more substantive methodological weaknesses (18).

The ERSPC (20) reported a significant 21% relative reduction (95%CI: 31%-8%) in prostate cancer-specific mortality in men aged 55-69 years, with the use of PSA screening, after 11 years of follow-up (20), while the PLCO study reported no significant effect (Risk Ratio (RR) for prostate cancer death=1.15, 95% CI 0.86-1.54) (21). The PLCO’s results are criticized due to the low compliance with biopsy in those with elevated PSA levels (31%) and due the contamination of the control groups by the performance of PSA testing in patients randomized for not being screened, which reflects the community practices in the United States; the contamination was estimated to be in the range of 53% and over 40% of men had undergone PSA testing before randomization, thus introducing a bias towards not finding a benefit of screening (18). In the ERSPC, the contamination rate was lower (≈ 20%) and the biopsy compliance was much higher (86%) (18).

The definitive diagnosis of prostate cancer depends on the histopathologic confirmation in prostate biopsy (19, 25).

In order to correctly refer patients to undergo prostate biopsy and to better assess the risk for prostate cancer, several risk calculators have been produced (26). Most of these tools consider, besides the PSA levels (27), DRE findings (28) and outcome of prior biopsy (26), the well-known risk factors for prostate cancer: age (29), family history (30) and race (31). However, the performance of these risk prediction tools depends on the cohort in which they were developed and on the PSA cut-off value used to recommend biopsy, since the relationship between PSA and risk of a positive prostate biopsy varies between cohorts (26).
3.2.1. Prostate Specific Antigen

PSA is a glycoprotein produced by the prostate gland and secreted in prostate lumen for liquefying the seminal fluid. In men without prostatic diseases, this protein is limited to prostate gland lumen. When any modification occurs in prostate physiology, PSA is released to the circulatory system, thus increasing the serum level (27).

This protein is specific for prostate but it is not specific for prostate cancer and several other prostate pathologies can also affect PSA level, including Benign Prostatic Hyperplasia (BPH), prostatitis or trauma (32). Although there is unquestionable evidence that prostate cancer risk varies with the levels of circulating PSA (27, 33), population-based screening programs are not recommended (18).

Guidelines differ among countries and medical organizations; the United States Preventive Services Task Force (USPSTF) recommend against routine widespread screening (34) as well as screening in men older than age 75 years, as these men present higher risk of adverse effects from treatment and competing causes of mortality making them less likely to benefit from screening (26, 34). Other organizations, like the American Cancer Society, the American and the European Urological Associations, present some recommendations to refer patients to undergo prostate biopsy, based on the presence of PSA serum levels above a specified cut-off and/or suspicious DRE (19, 25, 26). Physicians must also consider patients’ age, life expectancy, potential co-morbidities and the side effects of the prostate biopsy (19, 25).

The threshold of 4.0ng/mL has been traditionally used for prompting men to undergo prostate biopsy (19, 25). PSA testing has an overall sensitivity of 72.1% (66.7% to 100.0%), and a specificity of 93.2% (63.1% to 100.0%), when considering a serum PSA level higher than 4.0ng/ml, to define an increased risk of prostate cancer (35). The use of lower PSA threshold increases sensitivity while decreasing specificity, and no cut-off achieves both high sensitivity and high specificity (36).

To correctly identify the best PSA cut-off value and to increase its sensitivity and specificity, it may be necessary to account for some individual factors that affect PSA levels namely patients’ age (29), Body Mass Index (BMI) (37) and medications used (38).

Age

The use of age-specific cut-offs takes into account that, in men without prostate cancer, serum PSA reflects the amount of glandular epithelium, which in turn reflects prostate size; as prostate size increases with age, PSA concentration also rises (39). Therefore, in young patients, where almost all prostate cancer cases are clinical relevant and will lead to radical
treatment and the health costs of missing a diagnosis of prostate cancer are greater, a higher sensitivity is desired; in the older patients, in whom BPH is more likely to cause PSA elevation, there is higher probability of overdiagnosis, specificity becomes more relevant (40) which explains why, we found higher cut-offs for the older patients (41) (Table 1).

Table 1 – Age-specific reference ranges for serum PSA (42).

<table>
<thead>
<tr>
<th>Age range (yr.)</th>
<th>Reference range (ng/mL)</th>
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<tbody>
<tr>
<td>40-49</td>
<td>0.0 – 2.5</td>
</tr>
<tr>
<td>50-59</td>
<td>0.0 – 3.5</td>
</tr>
<tr>
<td>60-69</td>
<td>0.0 – 4.5</td>
</tr>
<tr>
<td>70-79</td>
<td>0.0 – 6.5</td>
</tr>
</tbody>
</table>

**Body Mass Index**

Several studies reported that increased BMI is associated with decreased serum PSA levels, due to a hemodilution effect, as a result of greater blood volume or due to suppression of PSA production caused by lower testosterone levels and higher oestrogen levels among obese men (37, 43). Lower PSA levels in obese men might decrease the sensitivity of prostate cancer screening, which would lead to a delayed diagnosis of prostate cancer, with an unfavourable prognosis (44, 45). However, the use of BMI-adjusted PSA cut-offs in prostate cancer screening is controversial and no specific ones have been developed (45).

**Medication**

There is increasing evidence over the impact of several therapeutic groups on PSA levels.

**5-α-reductase inhibitors**

5-α-reductase inhibitors (5-ARI) are used to treat BPH and have impact on prostate cancer due to the inhibition of the conversion of testosterone in dihidrotestosterone, which decreases prostatic volume and PSA levels (46-48).

It is suggested that in 5-ARI users it may be necessary to consider a PSA level that is twice the observed for the correct interpretation of the risk of cancer among these subjects (49). However, given the considerable inter and intra-individual variability in PSA levels, this
adjustment may overestimate actual PSA in some patients receiving 5-ARI for up to 6 to 9 months, accurately estimate PSA from 1 to 3 years and underestimate it among patients treated for more than 3 years (49). Instead, Marks et al recommends that the variation and the magnitude of the variation through time should be taken into account in patients under treatment with this therapeutic group, rather than a single PSA value (49). They also suggest the use of an increase in PSA of 0.3ng/ml or higher from the lowest value achieved with 5-ARI therapy (PSA nadir) as a trigger for biopsy; this maintains 71% sensitivity for prostate cancer in men receiving dutasteride, with 60% specificity, similar to the 4.0 ng/ml PSA cut-off using placebo (49).

Non-Steroidal Anti-inflammatory Drugs

There are reports of PSA levels 10% lower in Non-steroidal anti-inflammatory Drugs (NSAIDs) users when compared to non-users (50), and around 9% when comparing specifically aspirin with non-aspirin users (51, 52). The differences found might be confounded by the fact that men who use physician-prescribed NSAIDs – particularly aspirin, since it has preventive action over cardiovascular diseases – are more likely to be frequent users of other health services, such as cancer screening activities including PSA testing, causing a positive detection bias (52, 53).

Nevertheless, experimental studies have shown inhibitory effects of Non-steroidal Anti-inflammatory Drugs (NSAIDs) on prostate cancer cell proliferation and reduction of prostate cancer metastasis, suggesting their possible preventive role for prostate cancer (54) and NSAIDs also exert their activity on non-carcinogenic tissue, contributing to a reduction in serum PSA levels (51).

Thiazide diuretics

In a study of men over 40 years old without prostate cancer, users of thiazide diuretics presented 6% lower PSA levels after one year of therapy when compared to non-users (38). The results might be due to the depletion of vitamin D which is needed for the secretion of PSA and also due the induction of an androgen deficient state (androgens are needed for PSA production) (38).

Statins

Cholesterol is an essential molecule for the cell physiology; it is one of the main constituents of cell membranes, is responsible for the maintenance of membrane structural integrity and is also involved in cell signalling processes (55).

Several studies suggested that men with hypercholesterolemia are at increased risk for prostate cancer or late stage, aggressive disease (55-57). Moreover, prostate synthesises
cholesterol at a higher rate than the liver and it was found that cholesterol accumulation in prostate glandular tissue doubles further as the prostate progresses from benign to cancerous states (55). Therefore, perturbations on cholesterol metabolism are likely to influence cancer development and progression.

Statins are the most prescribed lipid lowering drugs within countries of the Organization for the Economic Co-operation and Development (OECD), with an estimated average daily consumption of 85.3 Defined Daily Dose (DDDs) per 1000 people (58). In 2011, in the USA, lipid regulators were responsible for a spent of 20.1$BN, with 19.8 million people treated with anti-cholesterol medicines (59). In Portugal, this therapeutic group represented the fourth most sold and around 9% of the total National Health Service medicines sales in 2011 (60).

The updates on the guidelines for cardiovascular disease prevention, with the definition of lower cut-offs for cholesterol levels associated with high cardiovascular risk, the recommendation for statins use in both primary and secondary prevention of cardiovascular events (61) and the intense marketing policies from pharmaceutical companies (62), contributed to the increasing use of statins over the last decades (63).

Statins act by inhibiting the Hydroxy-methyl-glutaryl Co-enzyme A (HMG CoA) reductase, responsible for the rate-limiting step in cholesterol synthesis – the conversion of hydroxyl-methyl-glutaryl in mevalonate (as depicted in Figure 1) (64).

**Figure 1** – The mevalonate pathway (adapted from Murtola *et al* (65))
The mevalonate pathway, in addition to the cholesterol synthesis, is also responsible for the biosynthesis of other metabolites like the isoprenoids geranyl-geranyl-pyrophosphate (GGPP) and farnesyl-pyrophosphate (FPP), which are lipid attachments that constitute key intermediates for a series of events essential for the activation and intra-cellular transport of cell-signalling proteins (64). These proteins act as molecular switches, controlling multiple pathways and cell functions such as maintenance of cell shape, motility, factor secretion, differentiation and proliferation (64). These effects, known as pleiotropic effects, are cholesterol-independent and include improvement of the endothelial function, inhibition of vascular inflammation and oxidation and stabilizing of atherosclerotic plaques (64, 66, 67).
3.3. Statins and serum PSA

In addition to the lipid lowering properties of statins, these drugs have shown anti-inflammatory, anti-invasive, tumour growth suppressing (68), apoptotic (69) and angiogenesis inhibiting properties (70) potentially decreasing the risk of several cancers (64).

Epidemiological evidence supports the association between statins therapy and overall cancer (71, 72) and reports significant reduced risk of specific cancer subtypes, namely liver cancer (RR=0.58, 95% CI: 0.46–0.74) (73), gastric cancer (Odds Ratio (OR)=0.70, 95%CI: 0.51-0.97) (74), esophageal cancer (OR=0.72; 95% CI: 0.60-0.86) (75) and reduced breast cancer recurrence among statins' users (RR=0.53, 95 % CI: 0.37-0.76) (76). Although it was not found an association between statins use and lung cancer, there are currently clinical trials testing the association of statins and anti-carcinogenic drugs, once statins might enhance the antitumor activity of various cytokines and chemotherapeutic agents specifically used in lung cancer treatment (77).

Dale et al (71), performed a systematic review of Randomized Control Trials (RCTs) of statins for cardiovascular prevention available until July 2005 and with information regarding cancer incidence and cancer deaths. They concluded that statins did not reduce the incidence of cancer (OR)=1.02, 95%CI: 0.97-1.07) or cancer deaths (OR=1.01, 95%CI: 0.93-1.09) (71). However some limitations were identified: the RCTs used in this study were designed to evaluate cardiovascular endpoints and, therefore, the definition of cancer as outcome might be limited; there were concerns about the patients' characteristics of those enrolled in the statin RCTs, since that, to maximize the effect of statins, the patients in these studies had a very high risk of cardiovascular disease events what represents a competing outcome for cancer diagnostic (71). Finally, if statins are chemo protective through their antioxidant properties, they may need to be given much earlier in life to be efficacious. It has been argued that antioxidants must be taken before significant oxidative damage has occurred to prevent cancer; therefore, the statin RCTs conducted might not have thoroughly examined the ability of statins to act as cancer chemo preventive drugs (71, 72).

Taylor et al (72), performed a meta-analysis of case-control studies that examined the relationship between any statin and neoplasm at any site. They found that the overall OR revealed a protective effect for statins of 0.71 (95%CI: 0.56–0.89) (72). The major weaknesses of this meta-analysis lied in the inherent potential biases of case–control studies, such as “recall bias” and “misclassification bias”. In addition, there could be other confounding factors that caused a false relationship to be identified between statins and all-cancers (72).

Prostate cancer is one of the cancer subtypes with evidence supporting a preventive role of statins over cancer incidence (78, 79).
3.3.1. Indirect effect

In a meta-analysis of both RCTs, with cancer as secondary endpoint, and observational studies, Bonovas et al., found a non-significant reduction on the risk of prostate cancer with the use of statins (pooled RR estimate: 0.95, 95%CI: 0.73-1.23) (78). However, in reports that specifically examined statins’ use and advanced prostate cancer, they found a protective association (RR: 0.77, 95%CI: 0.64-0.93) (78).

Bansal et al (79), updated Bonovas’ meta-analysis and included 17 more studies that were published after 2007. They found a significant inverse association between statin use and risk of total prostate cancer among cohort studies (RR=0.93, 95% CI: 0.87–1.01, p=0.09) and a non-significant inverse association among case-control studies (RR=0.87, 95%CI: 0.72–1.05, p=0.15) (79). The difference between these results and those presented by Bonovas et al. is likely to be due to the inclusion of 8 new studies that showed a negative association between statins and risk of total prostate cancer, as it was demonstrated by the cumulative meta-analysis performed (79). The reasons for this shift in the association might be the change in the behaviour regarding PSA testing, which lead to detection of early stage disease. Another explanation might be the fact that cholesterol levels in the pre-PSA era have a greater chance of being a product of tumour metabolism, leading to a positive association between statins and cancer, whereas cholesterol levels in post-PSA studies are more likely to reflect the cholesterol environment prior to the development of cancer (79).

There are several mechanisms that help explaining the association between statins’ use and prostate cancer, as presented in Figure 2, and they involve actions on cell cycle, apoptosis, angiogenesis and inflammation (80).
3.3.2. Direct effect

Statins may also contribute to a reduction in total PSA levels, due to their actions over benign prostatic tissue and over cholesterol metabolism – a precursor in the synthesis of PSA (80).

The first study that assessed the impact of statins on PSA values was performed on a small cohort of airline pilots diagnosed with hypercholesterolemia to whom statins were prescribed and a control group with normal cholesterol, followed up for a minimum of 15 months, from 1991 to 2002 (81). In this study, PSA declined an average of 42% over 5 years, and it raised the attention of the scientific community for the fact that this PSA decline could
represent further evidence of a preventive biologic effect of statins on the prostate and also for the fact that reduced PSA levels could lead to a delay on the referral for prostate biopsy, potentially causing missed or delayed prostate cancer diagnoses (81) (Figure 3). This delay causes a decrease risk of finding a localized tumour and an increased risk of finding advance tumours (79, 82). However, statins users are more likely to get PSA testing done, decreasing the time need to detect PSA levels high enough to trigger the diagnosis procedures, and this can be associated with an earlier detection of prostate cancer, leading to an increased risk of overall but a decreased risk of advanced prostate cancer (79, 82).

Figure 3 – Association between statins’ use and localized and advanced stage prostate cancer (adapted from Mondul et al (82)).

![Diagram showing association between statins' use and localized and advanced stage prostate cancer.]

- t₀ – development of asymptomatic tumour at same stage and grade for all scenarios
- PSA – PSA screening test when PSA concentration reached the level for referral for biopsy

After this study other observational investigations provide biologic evidence that statins reduce PSA levels (38, 82-86), but they were not able to ascertain the extent to which statins use influences PSA nor if there is the need to lower PSA threshold in statins’ users. The main characteristics and results of these studies are summarized in Table 2.
Table 2 – Main characteristics of the studies assessing the relation between statins and Prostate Specific Antigen (PSA) levels.

<table>
<thead>
<tr>
<th>Author et al. (83)</th>
<th>Year</th>
<th>Sample</th>
<th>Exclusion criteria</th>
<th>Main findings</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton et al.</td>
<td>2008</td>
<td>- computerized medical records at the Durham Veterans Affairs Medical Center of men who filled an outpatient prescription for a statin between January 1, 1990, and September 10, 2006 and with at least one PSA value within two years before and at least one PSA value within one year after starting a statin (n=1214)</td>
<td>- history/diagnosis of prostate cancer; - radical prostatectomy; - androgen deprivation therapy; - bilateral orchietomy; - transurethral resection of the prostate; - simple open prostatectomy; - taking finasteride; - taking testosterone; - prostatitis; - pre-statin PSA undetectable or &gt;10ng/mL - post-statin PSA undetectable; - not on statin at time of post-statin PSA; - taking a non-statin lipid lowering agent within 3 years before starting statin; - missing pre and post-statin LDL</td>
<td>- median PSA decline 4.1% [IQR – 22.1% to +12.5%, P&lt;0.001] per 214 days; - for every 10% LDL cholesterol decline after statin initiation, PSA declined by 1.64%</td>
<td>- exclusion of men with PSA≥10ng/mL; does not allow to comment on the association between statin use and PSA decline in these subgroups; - unable to capture information on possible lifestyle changes initiated by men who started statins, changes that could possibly explain the relationship between statins and PSA change. - full demographic data not available for all statin users, thus unable to assess if men included and excluded from analysis differed; - medication prescribed outside the Veteran’s Administration system was not captured. Thus, men may have started medications (e.g., finasteride) that potentially accounted for the observed PSA declines.</td>
</tr>
</tbody>
</table>

PSA – Prostate Specific Antigen
IQR – Interquantile Range
LDL – Low-density lipoprotein
Table 2 (Cont.) – Main characteristics of the studies assessing the relation between statins and Prostate Specific Antigen levels.

<table>
<thead>
<tr>
<th>Author et al (84)</th>
<th>Year</th>
<th>Population</th>
<th>Exclusion criteria</th>
<th>Main findings</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mener et al</td>
<td>2009</td>
<td>-computerized medical records at the University of Rochester Medical Center to identify men who filled statin prescriptions between May 31st and September 30th, 2008 and that underwent PSA testing within two years before and one year after starting statin therapy (n=962)</td>
<td>- history of prostate cancer, prostatitis, prior prostatectomy, transurethral resection of prostate, bilateral orchiectomy or androgen deprivation therapy; - treatment with finasteride or androgen supplementation.</td>
<td>- mean percent change: 8.04(-0.29ng/mL, 95% CI: -0.29,-0.22ng/mL) - mean follow-up time: 1.91 years</td>
<td>- not taking into account non-steroidal anti-inflammatory medication use during the study period; - statin use was evaluated based upon prescription data, and not actual compliance, which may overestimate statin use among patients. - evaluation of patients with prescribed statins for &lt;2 years and thus it may not allow for an observation of the peak reduction in serum PSA among patients.</td>
</tr>
</tbody>
</table>

CI – Confidence Interval
PSA – Prostate Specific Antigen
Table 2 (Cont.) – Main characteristics of the studies assessing the relation between statins and Prostate Specific Antigen levels.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Sample</th>
<th>Exclusion criteria</th>
<th>Main findings</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akduman et al (85)</td>
<td>2010</td>
<td>men from a prostate cancer screening population who had not taken any form of statin compared with the ones who had the indication for current use of a statin (n=4903)</td>
<td>N.A.</td>
<td>Statins’ use shown significant association with lower PSA (P=0.008), after adjusting for age, BMI and race</td>
<td>- unknown pre-stat-in serum PSA levels of patients receiving statin medication; does not allow to show the changes of serum PSA levels of these patients, and the relation of these changes to serum cholesterol changes. - unknown information regarding statin dose and compliance, and the effect of statin on LDL, HDL, and triglyceride.</td>
</tr>
</tbody>
</table>

BMI – Body Mass Index  
HDL – High-density lipoprotein  
LDL – Low-density lipoprotein  
N.A. – Non-applicable  
PSA – Prostate Specific Antigen
Table 2 (Cont.) – Main characteristics of the studies assessing the relation between statins and Prostate Specific Antigen levels.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Population</th>
<th>Sample</th>
<th>Exclusion criteria</th>
<th>Main findings</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murtola et al</td>
<td>2010</td>
<td>men participating in the Finnish Prostate Cancer Screening Trial from 1996 to 2004 (n=23,320)</td>
<td>- history of prostate cancer</td>
<td>- age-adjusted HR of prostate cancer; 0.62 (95%CI: 0.53-0.72); - multivariable adjusted HR: 0.75 (95%CI: 0.63-0.89) - age-adjusted mean PSA comparable between statins’ users and non-users; - men aged 60-72 who used statins had non-significantly lower PSA level compared to the ones not on statins</td>
<td>- current statin users who had a history of 6 months without medication purchases were reclassified to non-users until their next purchase, which may bias PSA level comparisons between the two groups towards the null; - 19.8% of men in the cohort used statin medications for less than 1 year, which may have been an insufficient amount of time to observe a substantial decrease in PSA.</td>
<td></td>
</tr>
</tbody>
</table>

HR – Hazard ratio
PSA – Prostate Specific Antigen
Table 2 (Cont.) – Main characteristics of the studies assessing the relation between statins and Prostate Specific Antigen levels.

<table>
<thead>
<tr>
<th>Author</th>
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<th>Sample</th>
<th>Exclusion criteria</th>
<th>Main findings</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mondul et al</td>
<td>2010</td>
<td>male participants in the 2001–2002 and 2003–2004 NHANES cycles, aged ≥40 years (n=2574)</td>
<td>- history of prostate cancer; - prostate biopsy or infection</td>
<td>-After multivariable adjustment for age, race/ethnicity, BMI and cigarette smoking status, men who reported currently using cholesterol-lowering drugs had a slightly lower PSA concentration (0.90ng/mL; 95%CI: 0.85-0.96) compared with nonusers (0.95; 95%CI: 0.91-0.99), although the difference was not statistically significant, P=0.20. - limiting the analysis to men without co-morbidities, the association between statins and PSA was stronger and statistically significant</td>
<td>- Cross-sectional nature that unable to longitudinally examine change in PSA concentration before and after statin use in the same man.</td>
</tr>
</tbody>
</table>

BMI – Body Mass Index
NHANES – National Health and Nutrition Examination Survey
PSA – Prostate Specific Antigen
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Sample</th>
<th>Exclusion criteria</th>
<th>Main findings</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Chang et al (38) | 2010 | men ≥40 years old without prostate cancer from the 2003 to 2004 and 2005 to 2006 cycles of the NHANES survey | - history of prostate cancer, prostatitis or recent prostate manipulation;           - inverse relationship between PSA levels and statin intake (P=0.01);  
- use of 5 ARI or other forms of hormone therapy;  
- incomplete medication, clinical or sociodemographic data                                                | - PSA levels after 1 year of statins were 3% lower, and after 5 years were 13% lower than those who weren’t taking medication  
- the relationship between statins and PSA is stable in men across a wide range of PSA values; |
|                 |      |                                                                        |                                                                                        |                                                                                                                                                        | - missing data in the NHANES dataset, may have been non-random, thus potentially resulting in non-responder bias;  
- cross-sectional nature of the study that precludes determination of a causal relationship between duration of medication use and differences in PSA levels;  
- unable to confirm the inverse relationship between PSA levels and NSAID, statin, and thiazide diuretic use substantially beyond 5 years, given the lack of men using the medications for more than 5 years |

5-ARI – 5-α reductase inhibitors  
NHANES – National Health and Nutrition Examination Survey  
NSAID – Non-steroid anti-inflammatory drugs  
PSA – Prostate Specific Antigen
4. OBJECTIVES

Prostate cancer is one of the main contributors to the high burden of cancer worldwide (6). It is the second most frequent cancer and the sixth cause of oncological death in men (1). Its incidence rates increased since the introduction of Prostate Specific Antigen (PSA) testing in the 1990s, but it have been stabilizing in the past decades, whilst mortality has been decreasing, mostly in developed countries (14).

PSA is a specific prostate gland glycoprotein, secreted into the circulatory system in case of any modification in prostate physiology – Benign Prostate Hyperplasia, prostatitis, trauma, cancer (27). Although it is not specific for prostate cancer, there is unquestionable evidence that the higher the PSA value, the more likely is the existence of prostate cancer (27).

The diagnosis of prostate cancer relies on the histopathologic confirmation of cancer in prostate biopsy (19). To refer for undergoing this invasive procedure, physicians must take into account digital rectal examination findings and serum PSA (19), interpreted according to patients' age (42), body mass index (43) and medications used (38), namely statins (87).

Statins are among the most prescribed anti-dyslipidemic drugs worldwide (58) and, in addition to the lipid lowering effects, the drugs belonging to this therapeutic group present anti-inflammatory, anti-invasive, tumour growth suppressing, apoptotic and angiogenesis inhibiting properties (64), potentially decreasing the risk of prostate cancer (79). Statins also contribute to a reduction in total PSA levels (81), reflecting the lowering of cholesterol levels, since it is a precursor in the synthesis of PSA, as well as due to their action on benign prostatic tissue (87).

The impact of statins on serum PSA levels may lead to a decrease and a delay in prostate cancer detection when using the traditional cut-offs for reference to biopsy, and adjustment of the PSA threshold to maximize sensitivity and specificity may be needed among statins' users. The present investigation has the following specific objectives, among patients referred to prostate biopsy:

1) To quantify the direct, indirect and total effects of using statins on PSA levels;

2) To assess the potential impact of the exposure to these drugs in the diagnostic accuracy of PSA.
5. MANUSCRIPT

5.1. USE OF STATINS AND SERUM LEVELS OF PROSTATE SPECIFIC ANTIGEN
Abstract

**Background**: Statins’ use has been associated with decrease risk of prostate cancer and with lower levels of serum total Prostate Specific Antigen (tPSA).

**Objective**: To quantify the effect of statins’ use on tPSA levels in patients referred to prostate biopsy. To determinate if the exposure to statins must be considered to improve the prostate cancer diagnostic accuracy of tPSA.

**Design, setting and participants**: We selected 551 patients referred to ultrasound guided trans-rectal prostate biopsy, with tPSA ≤10.0 ng/mL. Results of the prostatic pathology assessment (cancer versus non-cancer) were defined by biopsy. Information regarding statins’ use was obtained from patients’ clinical records.

**Outcome measure and statistical analysis**: We used path analysis to quantify the effect of statins’ use on tPSA and Receiver Operating Characteristic curves to assess the global predictive accuracy of models including tPSA, age, body mass index, 5-α-reductase inhibitors, aspirin and statins’ use for distinguishing between prostate cancer and benign conditions.

**Results and limitations**: We observed a negative total effect of statins on tPSA levels (β=-0.283; 95% CI: -0.618, 0.051), mostly due to the direct effect (β=-0.243; 95% CI: -0.587, 0.101) rather than the indirect effect (β=-0.040; 95%CI: -0.178, 0.097). There were no statistically significant differences between the area under the curve corresponding to the models with or without statins (P=0.554). The study is limited by its cross-sectional nature, and lack of information on the duration of treatment with statins. Results cannot be extrapolated to settings with a different risk of prostate cancer.

**Conclusions**: In a consecutive sample of patients referred to prostate biopsy, statins’ use contributed to lower tPSA levels, but the clinical impact in these patients is low.

**Patients Summary**: Among patients referred for prostatic biopsy, the current use of statins was associated with lower tPSA levels. However, the clinical impact in these patients is low.
Introduction

The use of statins has been increasing over the last two decades [1, 2] due to the definition of successively lower cut-offs for cholesterol levels associated with high cardiovascular risk [3], the changes in the spectrum of statins’ indication – with recommendations for its use in both primary and secondary prevention of cardiovascular events [3] – and the intense marketing policies by pharmaceutical companies [4, 5]. Currently, statins are among the most prescribed drugs within countries of the Organization for the Economic Co-operation and Development (OECD), with an estimated average daily consumption of 85.3 Defined Daily Doses (DDDs) per 1000 people [6].

In addition to the lipid lowering properties of statins, these drugs have shown anti-inflammatory, anti-invasive, tumour growth suppressing, apoptotic and angiogenesis inhibiting properties, potentially decreasing the risk of several cancers [7], including prostate cancer [7-9]. Statins may also contribute to a reduction in total Prostate Specific Antigen (tPSA) levels due to their action on benign prostatic tissue, in addition to the cholesterol lowering effects – a precursor in the synthesis of PSA [9]. Epidemiological evidence supports a lower risk of prostate cancer among statins users, and several observational studies have shown that non-cancer patients under treatment with statins, especially long therapy courses, present lower levels of serum tPSA [10-12].

The impact of statins on serum tPSA levels may lead to a decrease in prostate cancer detection when using the traditional cut-offs for reference to biopsy [11, 13], and adjustment of the tPSA threshold may be needed among statins’ users, to maximize sensitivity and specificity [14].

Therefore, we aimed to quantify the effect of using statins on tPSA levels, and to assess the potential impact of the exposure to these drugs in the diagnostic accuracy of tPSA, among patients referred to prostate biopsy.
Methods

Between October 2009 and November 2012, we consecutively recruited patients, at the Department of Urology of Hospital de São João, Porto, Portugal, referred to ultrasound guided trans-rectal prostate biopsy on the basis of abnormal digital rectal examination (DRE) or tPSA \( \geq 2.5 \text{ng/mL} \), and selected those with tPSA \( \leq 10 \text{ng/mL} \). Before undergoing prostatic biopsy, weight and height were measured and a fasting blood sample was collected for assessment of tPSA and free PSA (fPSA). None of the patients had received hormone therapy, radiotherapy or chemotherapy previously.

The results of the prostatic pathology assessment (cancer versus non-cancer) were defined by biopsy (12 to 14 biopsy cores). The participants with non-malignant disease in the first biopsy, but having a prostate cancer diagnosed within six months, were considered as cancer patients.

Information gathered by the physicians regarding the patients’ prostatic volume, previous medical conditions and currently used medication was obtained from clinical records for a total of 551 patients. Data analysis was restricted to this group.

Currently used medications were classified according to the Anatomical Therapeutic Chemical (ATC) classification system, from the 1\textsuperscript{st} level – Anatomical main group – to 5\textsuperscript{th} level – Chemical substance name) [15] – depending on the information available. Statins’ use was defined as the current treatment with any of the drugs belonging to ATC level C10AA (Hydroxy-methylglutaryl Coenzyme-A – HMG CoA – reductase inhibitors) including simvastatin (C10AA01), lovastatin (C10AA02), pravastatin (C10AA03), fluvastatin (C10AA04), atorvastatin (C10AA05), rosuvastatin (C10AA07) or pitavastatin (C10AA08). Statins were further classified as hydrophilic (pravastatin, rosuvastatin and pitavastatin ) or lipophilic (lovastatin, simvastatin, atorvastatin and fluvastatin). Data on specific dosage, duration of treatment or previously discontinued prescription medications were not systematically recorded and, therefore, not considered for analysis.

The Mann-Whitney and the Chi-Square tests were used to compare quantitative and categorical variables across groups, respectively. Statistically significance was considered when \( P<0.05 \). All reported \( P \)-values are two-sided.

To quantify the effect of statins’ use in tPSA levels, we used path analysis to account for the relations between variables depicted in the Directed Acyclic Graph (DAG) [16] presented as Figure 1. Path analysis is an extension of regression analysis which allows for simultaneous estimation of the interrelations between variables in a set [17]. The variables were chosen assuming a causal relation between statins and tPSA levels and taking into account the potential confounders. The model depicted reflects the minimum needed to estimate the global effect of statins on tPSA levels, including the direct (corresponding to the
influence on PSA biology and metabolism), indirect (through the relation with the risk of prostate cancer, which, in turn, is related to tPSA levels) and total effect (the net result of direct and indirect effects). Models were fitted with Mplus software (Muthe´n and Muthe´n, Los Angeles, California); 95% confidence intervals were calculated by bootstrapping and models were considered to have a good fit when the estimated Root Mean Square Error of Approximation (RMSEA) was 0.05 [18].

We fitted logistic regression models, including cancer as the dependent variable and different combinations of tPSA, age, BMI, 5-α reductase inhibitors (5ARI), aspirin and statins’ use as independent variables, due to their impact on tPSA levels, and used the areas under the corresponding Receiver Operating Characteristic (ROC) curves to compare their predictive accuracy to distinguish between prostate cancer and benign prostatic conditions [19]. The analyses were performed using STATA®, version 11.2 (StataCorp LP, College Station, TX, USA).
Results

There were a total of 149 statins’ users: 42.3% used simvastatin, 1.3% lovastatin, 18.1% pravastatin, 4.7% fluvastatin, 17.4% atorvastatin, 12.8% rosuvastatin and 0.7% pitavastatin. No significant differences were found between statins’ users and non-users, except for f/t PSA ratio among cancer patients, despite the tendency for statins’ users to be older and to have lower tPSA and fPSA levels, and f/t PSA (Table 1).

Figure 1 presents the relation between the variables tested to assess the plausibility of an effect of statins’ use on tPSA levels, using path analysis; the overall fit of the model was good (RMSEA=0.00). We observed a negative total effect of statins on tPSA levels (β=-0.283; 95% CI: -0.618, 0.051), mostly due to a direct (β=-0.243; 95% CI: -0.587, 0.101) rather than an indirect effect (β=-0.040, 95%CI: -0.178, 0.097). When considering specifically the use of lipophilic or hydrophilic statins, the former were more strongly associated with tPSA levels (total effect: β=-0.246; 95%CI: -0.610, 0.118 vs. β=-0.069; 95%CI: -0.465, 0.328).

Figure 2 depicts the ROC curves that reflect the global predictive accuracy of models including tPSA, age, BMI, 5ARI, aspirin and statins’ use for distinguishing between prostate cancer and prostate benign conditions. There were no statistically significant differences between the area under the curve (AUC) corresponding to the models with or without statins (P=0.554). Furthermore, there was no meaningful or statistically significant difference in the predicted probability of prostate cancer detection among statins users (24%; 95%CI: 17-31) and non-users (27%; 95%CI: 21-32) when setting the tPSA value to 4ng/mL and considering the mean values of the remaining variables included in the model.
Discussion

In a group of patients referred to prostate biopsy there was a negative non-significant effect of statins on tPSA levels, especially for lipophilic statins, and mainly due to a direct action on PSA synthesis and metabolism. However, taking into account the current use of statins did not affect meaningfully tPSA predictive accuracy for prostate cancer diagnosis. Furthermore, no important difference in the predictive value of PSA testing is to be expected according to statins use, since the probability of prostate cancer was similar among users and non-users.

Previous studies that evaluated the relationship between statins and tPSA levels found lower levels of tPSA among statins users [10-13, 20-22]; the relative difference in PSA levels between statins users and non-users was -3% and -13% after one and five years of therapy with statins, respectively, in a sample from participants in the National Health and Nutrition Examination Survey (NHANES) [13, 20]. However, methodological differences preclude direct comparison between our results and the ones from most studies conducted before.

Concerning the relationship between statins and cancer, we observed a non-significantly lower risk of prostate cancer, which is also in agreement with other studies that used a similar setting – men referred to prostate biopsy. In these studies, the risk ratios (RR) for prostate cancer diagnosis were 0.92 (95% CI: 0.85-0.99) [23] and 0.95 (95% CI: 0.73-1.24) [24] when adjusting for different confounders. Regarding the other relations depicted, the magnitudes of effects are in accordance with the available evidence with an exception for the association between aspirin use and tPSA levels [25, 26], since previous observational studies showed lower tPSA levels among aspirin users [13]. Regarding different statins grouped according to their solubility, our results agree with the hypotheses that lipophilic statins present greater intracellular access and therefore may be most effective in lowering PSA levels [7, 27].

Our study adds to previous research on this topic a comprehensive assessment of the impact of use of statins in prostate cancer; we quantified the statins direct (statins → tPSA) and indirect (statins → cancer → tPSA) effect – through path analysis – and assessed the impact of decreased tPSA levels among statins’ users in the prediction of prostate cancer – through ROC curve analysis. Nevertheless, some limitations need to be discussed. The absence of information from the patient’s clinical records regarding the duration of treatment with statins, since it is one of the main determinants for statins anti-carcinogenic activity [10, 13] contributes to an underestimation of the association between statins and prostate cancer and may help to explain why we observed a larger contribution from the direct effect on tPSA
levels. The cross-sectional nature of our analyses does not allow us to confirm the precedence of the exposure to statins over the observed tPSA levels. Although it is unlikely that there is a causal relation between low tPSA and the use of statins, the absence of information regarding access to health care services could have resulted in an overestimation of the direct effects of statins’ on tPSA levels [12, 26]. People who have a better access to health care are more likely to be prescribed a statin, possibly leading to lower tPSA levels, as well as to undergo tPSA testing more frequently and to be diagnosed with a prostate cancer on the basis of lower tPSA values. This probably had little impact in the internal validity of our findings, since all participants were referred for prostatic biopsy and a relatively high homogeneity in the access to health care may be expected among them. However, our results cannot be extrapolated to the general population or clinical settings with different risk of prostate cancer.

In conclusion, in a consecutive sample of patients referred to prostate biopsy we observed that statins’ use was associated with lower tPSA levels, but the clinical impact of this relation is low. Further investigation is needed to obtain more robust and generalizable estimates of the relation between the use of statins and tPSA levels.
<table>
<thead>
<tr>
<th></th>
<th>Non cancer (n=362)</th>
<th>Cancer (n=189)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non user (n=269)</td>
<td>User (n=93)</td>
<td>Non user (n=144)</td>
<td>User (n=45)</td>
</tr>
<tr>
<td></td>
<td>N*</td>
<td>Median (P25-P75)</td>
<td>p-value†</td>
<td>Median (P25-P75)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>551</td>
<td>65 (60-70)</td>
<td>66 (62-71)</td>
<td>0.057</td>
</tr>
<tr>
<td>tPSA (ng/mL)</td>
<td>551</td>
<td>5.45 (4.00-7.35)</td>
<td>5.30 (3.54-6.60)</td>
<td>0.323</td>
</tr>
<tr>
<td>fPSA (ng/mL)</td>
<td>542</td>
<td>0.89 (0.59-1.39)</td>
<td>0.85 (0.56-1.28)</td>
<td>0.328</td>
</tr>
<tr>
<td>f/t PSA</td>
<td>542</td>
<td>0.18 (0.14-0.24)</td>
<td>0.17 (0.13-0.24)</td>
<td>0.764</td>
</tr>
<tr>
<td>Abnormal DRE [n (%)]</td>
<td>159</td>
<td>61 (25.7)</td>
<td>18 (23.1)</td>
<td>0.638</td>
</tr>
<tr>
<td>Prostatic volume (mL)</td>
<td>472</td>
<td>59.5 (44-84)</td>
<td>60.0 (44-84)</td>
<td>0.996</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>551</td>
<td>26.4 (24.1-28.5)</td>
<td>26.6 (24.6-28.5)</td>
<td>0.461</td>
</tr>
</tbody>
</table>

P25-P75 – percentile 25-percentile 75  
BMI – Body Mass Index  
tPSA – total Prostate Specific Antigen  
fPSA – free Prostate Specific Antigen  
f/t PSA – free/total Prostate Specific Antigen ratio

* the number of participants may be lower due to missing data  
δ as appropriate  
† p-value for the comparison of statins' users vs non users
Figure 1 – Directed Acyclic Graph (DAG) representing estimates of direct (solid thick line) and indirect (solid dashed lines) effects of Statins on total Prostate Specific Antigen (tPSA) levels, calculated by path analysis*.

Direct effect (Statins → tPSA): $\beta_1 = -0.243 \, (-0.587; 0.101)$

Indirect effect (Statins → Cancer → tPSA) $\beta_2 \times \beta_3 = -0.040 \, (-0.178; 0.097)$

Total effect: $\beta_1 + (\beta_2 \times \beta_3) = -0.283 \, (-0.618; 0.051)$

* The regression coefficients ($\beta$) and 95% confidence intervals (95% CI) were obtained using linear (presented in italic) or logistic (bold) regression models, as appropriate.
Figure 2 – Receiver operating characteristic (ROC) curves for global predictive accuracy of different combinations of total PSA (tPSA), age, Body Mass Index (BMI), aspirin, 5-α reductase inhibitors (5ARI) and statins’ use for prostate cancer diagnosis.

AUC – Area Under the Curve
95% CI – 95% Confidence Interval
References


6. CONCLUSIONS

With this study, we were able to quantify the direct (statins $\rightarrow$ tPSA) and indirect (statins $\rightarrow$ cancer $\rightarrow$ tPSA) effects of statins using path analysis, and assessed the impact of decreased tPSA levels among statins’ users in the prediction of prostate cancer by comparing the Receiver Operating Characteristic curves, in a population of patients referred to prostate biopsy.

We concluded that the current use of statins, especially lipophilic statins, was associated with lower tPSA levels, mainly due to a direct action on PSA synthesis and metabolism, though this had little impact on the predictive value of PSA testing in this setting.
7. REFERENCES


60. INFARMED - Autoridade Nacional do Medicamento e Produtos de Saúde IP. Estatística do Medicamento. INFARMED, 2011


