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*in*

Melgarejo P. (ed.), Valero D. (ed.).  
II International Symposium on the Pomegranate

Zaragoza : CIHEAM / Universidad Miguel Hernández  
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 103

2012  
pages 311-320

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=6974>

To cite this article / Pour citer cet article

Chéchile Tonido G.E. **Usefulness of pomegranate in prostate cancer**. In : Melgarejo P. (ed.), Valero D. (ed.). *II International Symposium on the Pomegranate*. Zaragoza : CIHEAM / Universidad Miguel Hernández, 2012. p. 311-320 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 103)



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# Usefulness of pomegranate in prostate cancer

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**Abstract.** Pomegranate is the fruit of the *Punica Granatum* tree, which grows in Mediterranean countries. Its therapeutic properties have been used to treat different conditions (cardiovascular, neurological, diabetes and cancer) for hundreds of years. Numerous *in vitro* and *in vivo* studies have recently shown the antioxidant, anti-inflammatory and anti-tumoral properties of pomegranate. Antiproliferative, proapoptotic, antiangiogenic and inhibition of tumor invasion have been proven in several experimental models of urological tumors such as in prostate cancer. Pomegranate juice has been shown to delay PSA duplication time in patients with biochemical cancer recurrence after initial treatment. Many phase II studies are currently in progress to demonstrate the effectiveness of pomegranate juice in several diseases and in prostate cancer.

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## I – Introduction

The pomegranate, fruit of the *Punica granatum* tree, is native to the Himalayas in northern India, and Iran. Since antiquity its cultivation has spread to the Mediterranean countries, India, China, Japan and Russia, as well as areas of the United States and Afghanistan. The pomegranate's medicinal properties have been known for thousands of years, mention being made in the Old Testament of the Bible, the Jewish Torah, and the Talmud of Babylon. It was used in the ceremonies and mythology of the Ancient Egyptians, Greeks and Romans. Ayurvedic medicine regards the pomegranate as a pharmacy in its own right, using it against parasites, diarrhoea, diabetes, and to cure ulcers. In South America, pomegranate bark, peel and petals are chewed to treat dysentery, and mouth and gum problems<sup>1</sup>.

## II – Phytochemical constituents of the pomegranate

Phytochemicals are plant secondary metabolites having health-giving benefits, although they are not considered essential nutrients. Generally, phytochemicals are produced by the plant as part of its defence mechanism against external dangers, such as ultraviolet radiation, pathogens, etc<sup>2</sup>. Diets rich in phytochemicals are associated with a reduced risk of developing illnesses such as certain types of cancer, and inflammatory, cardiovascular or neurodegenerative diseases. Although the greatest source of the pomegranate's phytochemicals are found in the fruit, other parts of the tree, such as leaves and seeds also contain them. More than 100 phytochemical compounds have been isolated in the pomegranate. Those detected most frequently are the polyphenols, which include: a- *flavonoids* such as the anthocyanins and anthocyanidins (cyanidin, delphinidin, pelargonidin); b- *flavonols* such as luteolin, quercetin and kaempferol; c- *hydrolysable tannins* such as the ellagitannins, punicalagins and gallotannins. Hydrolysable tannins are responsible for 92% of the antioxidant activity of pomegranate juice and the punicalagins are responsible for half of this antioxidant effect<sup>3</sup>. Pomegranates also contain catechins such as those found in green tea, and steroids such as estradiol, estriol, estrone, testosterone and ursolic acid. The oil obtained from pomegranate seeds contains fatty acids, the most common of these being punicic acid (>60%). The structural variations between the polyphenols extracted from the various components –the fruit, juice or other parts of the pomegranate or the tree– are large.

## 1. The pharmacokinetics of pomegranate juice

Ellagitannins are hydrolysed rapidly in the body, becoming ellagic acid, of which no trace is found in the circulatory system after five hours<sup>4</sup>. Once absorbed, ellagic acid is metabolised by enzymes, such as glucuronosyltransferase and sulphotransferase, which increase its excretion and detoxification by increasing its water solubility. Intestinal microflora transform ellagic acid into two principal metabolites, urolithin A and B, which can remain in urine for up to three to four days after ingesting pomegranate juice; this may explain the beneficial effects of its chronic administration<sup>5,6</sup>. González-Sarrias *et al.* have shown the presence of urolithin A and traces of urolithin B in the prostate of men who had previously received pomegranate juice or walnuts for three days before surgery<sup>7</sup>.

## 2. Antioxidant effects of pomegranate

Recent research suggests that oxygen-dependent free radicals are the first step in physiopathological mechanisms of chronic illness and the aging process<sup>8</sup>. The increase of nitric oxide (NO) and nitric oxide synthase (NOS), associated with an excess in O<sub>2</sub> production, produces the formation of high levels of peroxynitrite (ONOO-)<sup>9</sup>. This compound causes direct toxic effects, such as lipid peroxidation, protein oxidation and DNA damage, as well as the induction of various transcription factors, including the nuclear factor kappa B (NF-κB) and the activator protein-1 (AP-1), which lead to cytokine-induced chronic inflammation. As a result of the latter mechanism, nitro-oxidative stress is transformed into an inflammatory process as these cytokines spread the inflammatory message via blood circulation, thus causing continuing cell damage (for example, endothelial cell dysfunction)<sup>10</sup>. DNA exposure to ONOO- or NO plus O<sub>2</sub> causes breaks in the chains<sup>11</sup>. Furthermore, the ONOO- renders inactive various enzymes that are important in repairing damaged DNA. Due to all these effects, ONOO- induces apoptosis if oxidation is moderate, or cell necrosis if oxidative stress is severe<sup>12</sup>. The antioxidant activity of pomegranate juice is three times higher than red wine or green tea<sup>13</sup>. Consuming 250 ml of pomegranate juice for four weeks has been proven to eradicate free radicals from the body, and significantly increase plasma antioxidant capacity in older people when compared to those consuming apple juice<sup>14</sup>. Rosenblat and Aviram proved that pomegranate juice contains a total higher concentration of polyphenols (5 mmol/l) and a greater antioxidant activity than other fruit juices (kiwi, apple, grape, orange, pineapple, pear, peach), which contain 1.3 – 4 mmol/l of total polyphenols<sup>15</sup>. These properties have a potential use as a complement in anti-aging treatment in both sexes.

## 3. Anti-tumour effects of pomegranate on prostate cancer

### A. Antiproliferative effects and proapoptosis

Several studies have shown that different parts of the pomegranate (arils, pericarp, seeds etc.), fresh or fermented, have antiproliferative effects. Albrect *et al*<sup>16</sup> showed *in vitro* that extracts derived from pomegranates inhibited the proliferation of several prostate cancer cell lines - hormone-sensitive (LNCaP) as well as hormone refractory (PC-3 and DU 145). However, normal prostate cells are not affected. Malik *et al*<sup>17</sup> assessed the antiproliferative effect and proapoptosis of pomegranate extract in aggressive hormone-refractory prostate cancer cells (PC-3), and observed a dose-dependent inhibition in cell-growth and apoptosis induction. This effect came about due to the decrease in expression of the anti-apoptotic Bcl-2 gene protein and the increase in expression of the pro-apoptotic Bax gene protein. In one *in vivo* experiment in which athymic mice were implanted with hormone-sensitive prostate cancer cells, tumour growth was observed to be slower in animals that had been administered with pomegranate extract as their sole source of liquid compared to those drinking only water. Furthermore, the animals receiving pomegranate extract showed a significant reduction (up to 85%) in PSA production<sup>23</sup>. Seeram *et al*<sup>18</sup> obtained similar results from pomegranate juice in respect to growth inhibition of prostate cancer cells *in vitro* and *in vivo*. They also observed that the

uroolithins (ellagic acid metabolites) were localised in the prostate, inhibiting growth of both hormone-sensitive and hormone-refractory cancer cells. Recently, Koyama *et al* have shown that pomegranate juice induces apoptosis in prostate cancer cells by inhibition of IGF<sup>19</sup>. These results suggest that consuming pomegranate may delay the growth of prostate cancer, which could lengthen and improve patients' quality of life.

### **B. Effects on the nuclear kappa B (NF-κB)**

NF-κB forms part of a family of transcription factors and is activated as a response to various stimuli: cytokines, carcinogens, chemotherapies, endotoxins, chemical or physical stress, radiation, hypoxia, and inflammation. Activated NF-κB is found in several tumours and it has been shown to regulate the expression of more than 200 genes with different functions that participate in regulating the immune system, carcinogenesis, cell proliferation and adhesion, anti-apoptosis, angiogenesis, invasion and metastasis<sup>20</sup>. NF-κB activity is regulated by an inhibiting protein that binds onto it, retaining it in the cytoplasm. When the NF-κB route is activated, the inhibiting protein degrades by phosphorylation, releasing NF-κB, which translocates to the nucleus where it acts as a transcription factor<sup>21</sup>. Prostate cancer is one of the tumours in which NF-κB activation has been shown to be present and represents an independent risk factor of tumour recurrence after radical prostatectomy<sup>22,23</sup>. Rettig *et al* have demonstrated *in vitro* that pomegranate juice, as well as pomegranate extract, inhibit NF-κB and cell viability in prostate cancer cells. In one *in vivo* model, pomegranate was seen to delay the appearance of hormone resistant prostate cancer<sup>24</sup>. NF-κB inhibition is a necessary mechanism to obtain the maximum pro-apoptotic effect from pomegranate juice.

### **C. Effects on angiogenesis**

Hypoxia is the principal mechanism in the progression of more than 70% of tumours via the activation of angiogenesis, an essential factor for a tumour to be able to grow more than 200 micras<sup>25</sup>. However, in contrast to what happens with the vascularisation of normal tissue, the tumoural microvessels formed through angiogenesis are very disorganised. Thus greater hypoxia is produced with the subsequent activation of transcription factors associated with cellular hypoxia, such as hypoxia-inducible factor 1-α and 1-β (HIF-1α and HIF-1β); these in turn activate different genes related to angiogenesis, leading to greater progression and invasion<sup>26</sup>. Tumour-induced angiogenesis is regulated by factors produced by macrophages, neutrophils, and by the tumoural cells themselves as the vascular endothelial growth factor (VEGF). In prostate cancer, for example, it has been shown that androgens, which play an important role in tumour aetiology and progression, activate the expression of HIF-1α and VEGF<sup>27</sup>. Toi *et al* analysed the anti-angiogenesis potential of pomegranate seed oil or fermented pomegranate juice on estrogen-sensitive (MCF-7) or estrogen-resistant (MB-MDA-231) breast cancer cells, observing a significant reduction<sup>28</sup>. Sartippour *et al* carried out *in vitro* studies on the effect of pomegranate peel extract (standardised to 37% ellagitannins and 3.5% free ellagic acid) on hormone-sensitive prostate cancer cells (LNCaP) and human umbilical vein endothelial cells<sup>29</sup>. Pomegranate extract inhibited the proliferation of the endothelial cells under both normoxic and hypoxic conditions, and inhibited the proliferation of LNCaP cells under hypoxic conditions. Under hypoxic conditions, a reduction was also observed in the concentration of HIF-1α protein and VEGF in both cell groups. In an *in vivo* experiment, human prostate cancer cells (LAPC4) were implanted into mice with severe combined immunodeficiency (SCID); the animals then received either pomegranate extract or a liquid serving as control by mouth five days a week for four weeks. The pomegranate extract the animals received was the equivalent of a human intake of 320 ml of pomegranate juice. After four weeks, tumour volume was observed to be significantly smaller (199±37 mm<sup>3</sup> compared to 1179±106 mm<sup>3</sup>) in those animals that had received the pomegranate extract. Furthermore, VEGF concentration was significantly higher in those animals receiving the control liquid, while HIF-1α staining and blood vessel density were reduced significantly in those animals receiving pomegranate extract<sup>29</sup>.

#### **D. Effects on tumoural invasion**

For tumours to be able to infiltrate surrounding tissue, tumour cells need to secrete proteolytic enzymes, such as metalloproteinases, in order to digest the extracellular matrix. Pomegranate extract has been proven to be effective in inhibiting metalloproteinase expression by inhibition of NF- $\kappa$ B in human chondrocytes<sup>30</sup>. In another study, several constituents of pomegranate (ellagic acid, caffeic acid, luteolin and punicalic acid) were examined *in vitro* for their potential inhibiting effect on human hormone-refractory prostate cancer cell (PC-3) invasion through an artificial membrane<sup>31</sup>. Although each of the substances separately significantly inhibited invasion, when used together, a supra-additive effect was seen. Albrecht *et al* had similar results with the same type of prostate cancer cell<sup>16</sup>.

### **III – Prostate cancer epidemiology**

If non melanoma skin cancer are excluded, prostate cancer in Spain is the third tumour in frequency after lung and colorectal cancer. In Spain one in six males will develop prostate cancer during his life. The probability to develop prostate cancer increases with the age, since one in each ten cases are diagnosed in men above 65 years old. In relation with mortality, in Spain, prostate cancer is the third tumour after lung and colorectal cancer. Since 1998 prostate cancer has decreased in some regions like Madrid, Cataluña, Valencia and Baleares, while in others regions the incidence of prostate cancer is increasing. In Europe, prostate cancer causes 3% of all deaths in males. Scandinavian countries, Belgium, Netherlands and some regions of France have a high mortality meanwhile Bulgaria, Hungary, Romania and Mediterranean countries mortality due to prostate cancer is below the mean. Mortality in Spain is between the lowest as Italy and Greece. Nowadays less than 5% of the patients with prostate cancer present metastasis in the moment of diagnosis in front of 50% in the 80's decade.

#### **1. Prostatic specific antigen (PSA)**

Prostate-specific antigen (PSA) is a protein produced specifically by cells of the prostate gland. It is normal for men to have a low level of PSA in their blood; however, prostatic diseases like prostate cancer, benign prostatic hyperplasia or prostatitis can increase a man's PSA level. It is a very useful tumour marker. In patients with prostate cancer treated with radical prostatectomy serum level of PSA should be undetectable (<0,04 ng/ml). Increase of PSA level after surgery or radiotherapy means tumour recurrence.

#### **2. Biochemical recurrence**

If recurrent prostate cancer is detected by a rise in PSA levels after curative treatment, it is referred to as a "biochemical recurrence". In patients treated by radical prostatectomy the increase of PSA above 0,20 ng/ml is considered as biochemical recurrence. If it is observed before two years of surgery is considered a bad prognosis. In 37% of the patients with biochemical recurrence metastasis will be seen before 8 years and they will die in 5 more years. The likelihood of developing recurrent prostate cancer after curative treatment is correlated to various risk factors, such as the grade of prostate cancer (Gleason score), PSA level prior to treatment, and the stage of disease prior to treatment. Patients with low-grade cancer (Gleason score  $\leq$  6), PSA < 10, and tumors that are not palpable by digital rectal examination are at the lowest risk of recurrence.

#### **3. Treatment of biochemical recurrence**

Biochemical recurrence after radical surgery can be treated with external radiotherapy or androgen deprivation. Radiotherapy should be started when PSA reach 0,50 ng/ml. La radioterapia externa debe iniciarse cuando el PSA alcanza el valor de 0,50 ng/ml ya que las

posibilidades de curación del cáncer son mayores que si se espera que el PSA alcance valores superiores. Patients treated with external beam radiotherapy as a primary treatment of prostate cancer only can receive hormonal therapy in case of recurrence. Any treatment than delay biochemical recurrence will avoid progression of cancer

#### 4. PSA double time (PSADT)

Doubling time is defined as the duration for PSA levels in the blood to increase by 100 percent. A longer PSADT is associated with a longer time to metastasis, to prostate cancer-specific death, and to death from all causes. Furthermore, as the PSADT increases, so do the time to metastasis and the time to prostate cancer-specific death. Men with a PSADT of < 3 months are at high risk for adverse clinical outcomes. Men with a PSADT of > 15 months have an extremely low risk of death from prostate cancer. For men with a PSADT between 3 and 15 months, other clinical factors may have a larger role in determining risk.

### IV – Clinical applications of pomegranate juice in prostate cancer

All parts of the pomegranate have been used to treat a variety of illnesses for over a thousand years. However, it was not until the early 90s that the first experimental and clinical trials began<sup>1</sup>. Pantuck *et al*<sup>32</sup> undertook a phase II clinical trial with 46 men with prostate cancer who had been treated by surgery, radiotherapy or criotherapy, and whose PSA levels had increased. The inclusion criteria were a Gleason score of  $\leq 7$  and PSA between 0.2 and 5 ng/ml. Treatment consisted of 240ml of pomegranate juice a day until the illness progressed. None of the patients had metastasis nor had they received hormonal treatment. Follow-up was carried out every three months and PSA levels determined. The aim of the investigation was to study the variation in PSA figures, such as doubling time. Concurrently an *in vitro* study of cell proliferation was undertaken in which patients' serum was incubated with a culture of hormone-sensitive prostate cancer cells (LNCaP). Of the 46 patients, 16 (35%) showed a reduction in PSA values. In four cases PSA dropped by more than 50%. PSA doubling time (PSADT) increased significantly, from an average of 15 months at the beginning of the study up to 54 months ( $p < 0.001$ ). In the *in vitro* study, after nine months a 12% reduction in prostate cancer cell proliferation was observed, and a 17% increase in apoptosis. Results from the patients who had continued the treatment with pomegranate juice were presented at the 2008 Annual Congress of the American Society of Clinical Oncology (ASCO), the findings showing that PSADT increased at 68 months<sup>33</sup>. These results suggest that pomegranate juice is effective in delaying the progression of prostate cancer in patients whose initial therapies had been unsuccessful. Carducci *et al* presented at 2011 Annual Meeting of ASCO the results of a randomized, multi-center, double blind, Phase II clinical trial that compared two different doses of pomegranate capsules (POMx) in men with a rising PSA after primary treatment who wished to delay starting androgen deprivation therapy (ADT)<sup>34</sup>. The trial randomized men who had a rising PSA but no metastases. The men received either high-dose (3 mg/d) or low-dose (1 mg/d) of POMx. The men were stratified based on their baseline PSADT values and their Gleason scores; however, there were no restrictions on PSADT and no upper limit PSA scores. The end points were until disease progression or for 18 months. PSA scores were recorded every 3 months. This study was designed to detect a 6-month increase in PSADT from baseline. The results of the study, as presented in the study abstract, are as follows: a- 104 men were enrolled and treated for up to 6, 12, and 18 months (92, 70, and 36 percent of men respectively); b- the men had a median age of 74.5 years, and a median Gleason score of 7; c- the average (median) PSADT was 11.9 months at baseline with a range of 1.6 to 54.6 months compared to 18.5 months with a range of 2 to 1.523 months after treatment ( $p < 0.001$ ); d- there was no significant treatment difference in effect on PSADT between the two dose groups ( $p = 0.920$ ); e- declining PSA levels were observed in 13/104 men (13 percent) during the study; f- there were no significant changes in serum testosterone levels in either group; g- mild to moderate diarrhea was seen in 8/104 men (7.7 percent). This study concludes that

pomegranate extract has some clinically positive effect on the PSADT. It also shows that the lower dosage of POMx is equally as effective as the higher dosage. The authors concluded that "POMx treatment significantly increased the PSADT by over 6 months in both treatment arms, with no effect on testosterone."

## 1. Our experience with pomegranate on prostate cancer

Progressive increase of PSA is the natural evolution when biochemical recurrence is diagnosed in patients treated initially with radical prostatectomy. The objective of pomegranate treatment is to stop or delay cancer progression.

We are treating with pomegranate 30 patients with prostate cancer. Ten of the 30 cases are patients with increase of PSA after radical prostatectomy (between >0.04 and 0.50 ng/ml). Surgery had been performed between 1 and 132 months before (mean 35 months). Pomegranate treatment range from 1 to 30 months (mean 13 months). During follow up, PSA was measure each 3-6 months. Mean age was 68 years old (54-73 years). Three cases were followed  $\geq 24$  months, 6 cases 12 months, 7 cases 6 months and 5 cases 3 months. At 3 months PSA decrease in 3/5 cases (from 0.27 to 0.21, from 0.20 to 0.17 and from 0.09 to 0.08 ng/ml). In one case there was no changes and in one case PSA increases (from 0.20 to 0.31 ng/ml). At 6 months PSA decrease in 3 patients (from 0.31 to 0.15, from 0.21 to 0.16 and from 0.24 to 0.16 ng/ml), and in 4 patients there were no changes. At 12 months PSA decrease in one patient (from 0.20 to 0.04 ng/ml), in 4 patients there were no changes and in one case increase (from 0.11 to 0.16 ng/ml) but without reaching the level of biochemical recurrence (0.20 ng/ml). At 24 months, in one patient there was no change and in two cases PSA increases from 0.04 to 0.20 and from 0.16 to 0.20 ng/ml). Treatment with pomegranate was well tolerated without severe adverse effects. At the time of final review any patient treated with pomegranate have required treatment with radiotherapy because PSA is below 0.50 ng/ml.

## 2. Clinical cases

### A. Case 1. Delay disease progression

70 years old male. Initial PSA 11 ng/ml. Radical prostatectomy on May 2007 for adenocarcinoma Gleason 3+4, stage pT2c. Start pomegranate on June 2009. 2009.

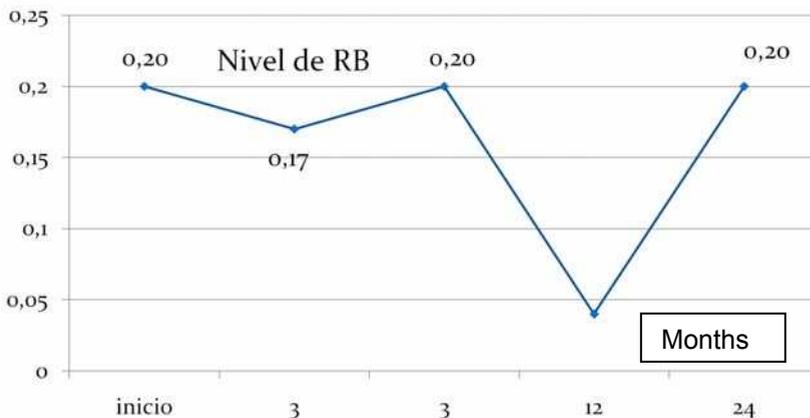


Fig. 1. PSA variation after 24 months of treatment with pomegranate.

### B. Case 2. Delay of biochemical recurrence

67 years old male. Initial PSA was 6,08. Radical prostatectomy was performed on October 2007 for Gleason 3+4 adenocarcinoma. Pomegranate was started on October 2008.

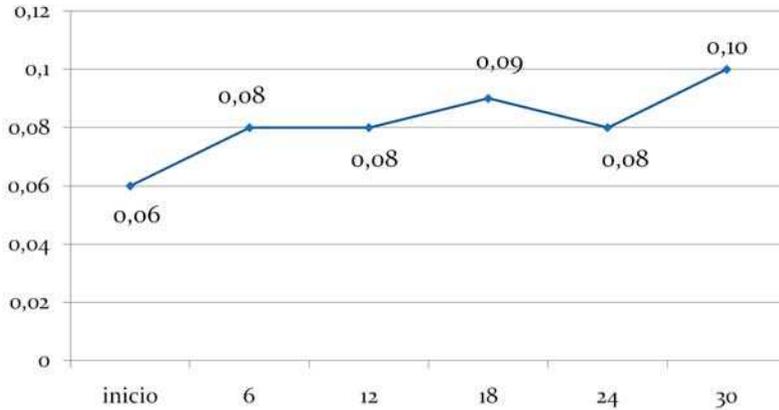


Fig. 2. Variation of PSA after 30 months of treatment with pomegranate. Biochemical recurrence was delayed.

### VARIACION DE PSA

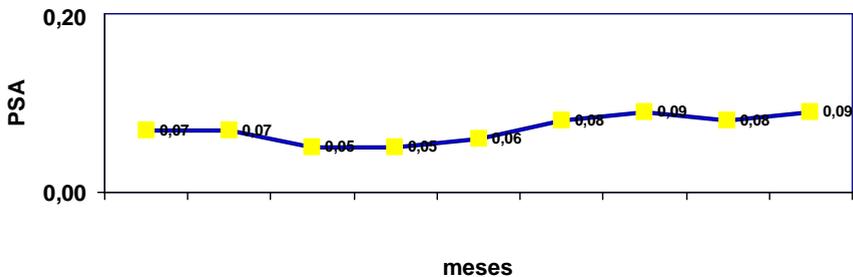


Fig. 3. Delay of hormonal treatment after failure of two treatments for próstate cancer.

### C. Case 3. Delay to start hormonal treatment

73 years old male. Initial PSA was 4,16 ng/ml treated with external beam radiotherapy in 2000, hormonal treatment until September 2003. PSA increase from 2003 and 2006. High intensity focalizad ultrasound (HIFU) was performed on March 2007. PSA was undetected until March 2009 and later it increased. Pomegranate juice was started at this time.

## VARIACION PSA

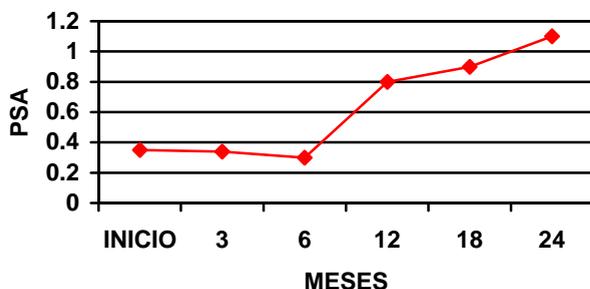


Fig. 3. PSA variations after 24 months of treatment with pomegranate. Hormonal treatment have been delayed during 2 years.

### 3. Ongoing clinical trials using pomegranate juice on prostate cancer

Nine of the investigations are related to prostate cancer, six of which are recruiting participants with high PSA levels after the failure of the initial treatment with radical prostatectomy or radiotherapy. In another study patients are treated with pomegranate juice before undergoing radical surgery. Another trial treats patients with localised prostate cancer who have not yet received any treatment, and the final study evaluates supplementing prostate cancer patients' diets with phytochemicals and polyunsaturated fatty acids.

## V – Conclusions

The properties of the pomegranate have been known for more than a thousand years, however, it has only been in the last couple of decades that the number of *in vitro* and *in vivo* trials analysing its various components (especially the juice) and their effect on different pathologies has increased. Likewise, over the last few years several multi-centre clinical trials have been designed and are currently in progress; when their results have been analysed, they will be able to offer us a great deal of information about the therapeutic effects of pomegranate. For the time being, its potent antioxidant activity, similar to or greater than green tea, has been proven; it could thus be used as an adjuvant in anti-aging treatments. In oncology, its antiproliferative, pro-apoptotic and angiogenesis effects have been widely studied in animal models and are pending confirmation from human studies. The capacity of pomegranate to regulate plasma levels of glucose, cholesterol and triglycerides, and to reduce blood pressure opens an ample therapeutic potential for patients with diabetes and cardiovascular disease. The possible use of pomegranate juice in other fields, such as neurology and contagious diseases, needs further research.

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