CTM Cancer Translational Medicine

Cancer Transl Med 2016;2(4):105–12
doi: 10.4103/2395-3977.189302

Original Article

Impact of Age on the Biochemical Failure and Androgen Suppression after Radical Prostatectomy for Prostate Cancer in Chilean Men

Nigel P. Murray[1,2], Eduardo Reyes[1,3], Nelson Orellana[1], Cynthia Fuentealba[1], Omar Jacob[1]

[1]Urology Service, Hospital de Carabineros de Chile, Nunoa, Santiago, Chile; [2]Faculty of Medicine, University Finis Terrae, Providencia, Santiago, Chile; [3]Faculty of Medicine, University Diego Portales, Santiago, Chile

Address for correspondence: Dr. Nigel P. Murray, Faculty of Medicine, University Finis Terrae, Avenida Pedro de Valdivia 1509, Santiago, Chile. E-mail: nigelpetermurray@gmail.com

Received April 13, 2016; Accepted August 10, 2016

Aim: The aim of this study was to determine the association of age with the clinicopathological findings, phenotypic expression of circulating prostate cells (CPCs) and micrometastasis, biochemical failure and effect of androgen blockade after radical prostatectomy for prostate cancer in Chilean men.

Methods: This is a prospective, observational, single-center study of Chilean men who underwent radical prostatectomy. Three months after surgery, the peripheral blood was collected to analyze the biochemical failure by measuring prostate-specific antigen (PSA) levels in the serum. The blood and bone marrow were collected to detect the presence of CPCs and the bone marrow micrometastasis by checking the expression of PSA, human epidermal growth factor receptor 2 (HER-2), and matrix metalloproteinase 2 (MMP-2) using standard immunocytochemistry. The clinicopathological findings, phenotypic expression of CPCs and micrometastasis, biochemical failure and effect of androgen blockade were analyzed for association with age.

Results: In total, 120/338 (36.6%) of patients were ≥ 70 years (older men). A higher frequency of biochemical failure occurred in older men with negative surgical margins, a Gleason score ≥ 8, and pT3 tumors compared to patients < 70 years of age (younger men). The expression of HER-2 and MMP-2 was higher in CPCs and micrometastasis in older men. After androgen blockade, the expression of HER-2 and MMP-2 was similar in both groups. With androgen blockade, more younger men became micrometastasis negative (49% *vs.* 15%) while more older men became castrate resistant (83% *vs.* 43%).

Conclusion: After radical prostatectomy, the older men with pathological features of Gleason score ≥ 8, pT3 tumors, and positive extracapsular extension had higher frequency of biochemical failure and the presence of CPCs. The treatment of androgen blockade was less successful to suppress the disease relapse in the older men than that in the younger man.

Key words: Androgen blockade, biochemical failure, circulating prostate cells, prostate cancer, radical prostatectomy

INTRODUCTION

The 2015 National Comprehensive Cancer Network guidelines[1] recommend definitive treatment for prostate cancer in men with a life expectancy of > 10 years, with radical prostatectomy as a standard treatment option.[2,3] It has been reported that the frequency of adverse risk factors for treatment failure is increased in men older than 70 years, and this is associated with a higher risk of biochemical failure.[2,3] However, other studies have failed to show this association.[4,5] As such, it is unknown whether age itself is a biological parameter of prostate cancer aggressiveness or if it is a result of adverse pathological characteristics due to a later diagnosis.

How to cite this article: Murray NP, Reyes E, Orellana N, Fuentealba C, Jacob O. Impact of age on the biochemical failure and androgen suppression after radical prostatectomy for prostate cancer in chilean men. *Cancer Transl Med* 2016;2(4):105-12.

Considering these facts, we assessed the impact of age on the risk of biochemical failure after radical prostatectomy and analyzed the biological features of patients with bone marrow micrometastasis detected after the initial treatment. Secondary circulating tumor cells and bone marrow micrometastasis, each with different biological characteristics compared with the primary tumor, have been shown to increase the risk of biochemical failure.[6-11] These characteristics may differ in different age groups and require different therapy strategies. Two biomarkers' expressions, human epidermal growth factor receptor 2 (HER-2) and matrix metalloproteinase 2 (MMP-2) expressions, were analyzed in circulating prostate cells (CPCs) and micrometastasis, both of which have been reported to be associated with disease aggressiveness.[11-14]

HER-2 is a member of the ErbB family of tyrosine kinase receptors, which is thought to play a crucial role in the growth, differentiation, and motility of cancer cells. In the absence of androgens, HER-2 has been proposed as a survival factor for prostate cells, possibly by activating the androgen receptor.[12,15,16] In *in vitro* cell line model studies, HER-2 expression is found elevated in castrate resistant tumors and shows early involvement in the androgen dependence to independence switch.[17,18] The proposed mechanism in this switch is that the HER-2 receptor activates the androgen receptor by phosphorylation via the MAPK or AKT pathways and thus maintains the integrity of the androgen receptor and its function in the absence of testosterone.[19,20] The expression of HER-2 in bone marrow micrometastasis has been reported to be similar in men with and without biochemical failure; however, with androgen blockade, the frequency of HER-2 positive men increased, implicating that HER-2 positive cells are resistant to androgen blockade and are selected in an androgen-depleted environment.[13,21] The overexpression of HER-2 increases the production of MMP-2 by upregulating the transcription and activity of promoter – MMP-2 via MAPK and P13K via androgen receptor activation.[22,23]

MMP-2 expression in primary prostate cancer is associated with worse prognosis and is thought to be important in the dissemination and invasion of cancer cells.[14,24-27] MMP-2 activates MMP-9 which is known to activate angiogenesis and thus promotes the growth of both tumor and metastatic tissue.[28]

The objective of this study was to determine the association of age with the clinicopathological findings, phenotypic expression of CPCs and micrometastasis, biochemical failure and effect of androgen blockade after radical prostatectomy for prostate cancer in Chilean men.

METHODS

The study was approved by the Local Ethics Committee and was conducted in complete agreement with the "Declaration of Helsinki." All patients provided written informed consent. The study was designed as a single-center, prospective, observational study involving consecutive prostate cancer patients who underwent radical prostatectomy. The age, pretreatment serum total prostate-specific antigen (PSA), Gleason score, pathological stage, extracapsular extension, infiltration of the seminal vesicles and lymph nodes as well as positive surgical margins, defined as one with cancer cells in contact with the inked surface of the specimen, were recorded.

Three months after surgery, patients had their blood and bone marrow analyzed to detect PSA-expressing CPCs and micrometastasis, and the positive cases were classified according to their immunocytochemical staining for HER-2 and MMP-2. Patients were then followed up for serial total PSA levels, every 3 months for the 1st year and every 6 months thereafter.

Biochemical failure was defined as serum PSA level of > 0.2 ng/mL; readings were taken on two occasions separated by at least 2 weeks. Men who suffered biochemical failure were treated with flutamide 250 mg t.d.s for 4 weeks and leuprorelin 11.25 mg depot injection every 3 months until disease progression, defined as a rise in PSA on two occasions separated by at least 2 weeks. Under androgen blockade, the biochemical failure cases underwent blood analysis for CPC detection and bone marrow evaluation for micrometastasis (median observation time for progression was 18 months). Patients treated with androgen blockade not showing a rise in PSA were reevaluated at the end of the 2-year study period [Figure 1].

Exclusion criteria

Men who had received or were receiving androgen blockade at the beginning of the study, as well as men presenting with metastatic disease, as defined by a positive bone scan, were excluded from the study.

Detection of secondary circulating prostate cells

An 8 mL of venous blood sample was collected in an ethylenediaminetetraacetic acid containing vacutainer (BD-Vacutainer®). Samples were maintained at 4°C and processed within 48 h. CPC detection was independently evaluated with the evaluators being blinded to the clinical details.

Collection of circulating prostate cells

Mononuclear cells were obtained by differential centrifugation using histopaque 1077 (Sigma-Aldrich), washed, and resuspended in 100 µL aliquots of autologous plasma. Moreover, 25 µL aliquots was used to make slides (Silanized, DAKO, USA); dried in air for 24 h; fixed in a solution of 70% ethanol, 5% formaldehyde, and 25% phosphate-buffered saline (PBS), pH 7.4, for 5 min; and finally washed three times in PBS.

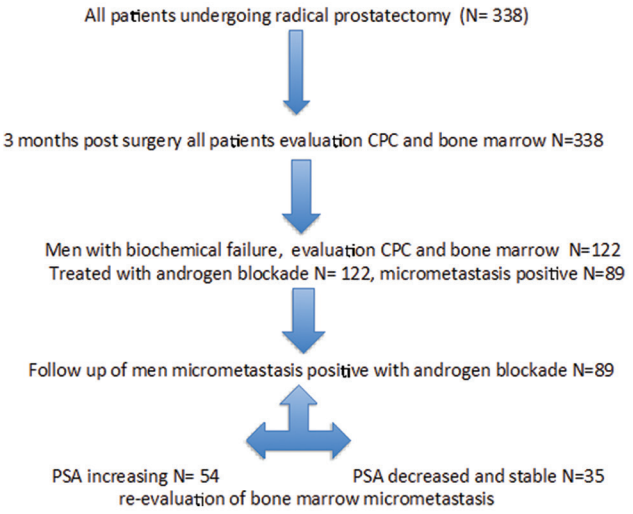

Figure 1. Flowchart of patient follow-up

Immunocytochemistry

Secondary CPCs were detected using a monoclonal antibody directed against PSA, clone 28A4 (Novocastra Laboratories, UK), and identified using an alkaline phosphatase – anti-alkaline phosphatase-based system (LSAB2, DAKO, USA), with new fuchsin as the chromogen. A test was considered positive for secondary CPCs when at least one cell positive for PSA/8 mL of blood was detected. The number of CPCs detected/8 mL blood sample was registered. Positive samples underwent a second staining process where half of the slides were stained for MMP-2 and the other half for HER-2. For MMP-2 staining, the slides were incubated with anti-MMP-2 clone 1B4 (Novocastra Laboratories, UK), for 1 h, and identified with a peroxidase-based detection system (LSAB2, DAKO, USA) with 3,3 diaminobenzidine tetrahydrochloride hydrate as the chromogen. The sample was defined positive or negative for MMP-2 expression based on the Trudel's criterion to define a cell expressing MMP-2.[14] Staining of MMP-2 was membranal in location.

For HER-2 staining, following manufacturer's instructions, the slides were incubated with anti-HER-2 using the HercepTest® (DAKO, USA). A sample was considered positive for HER-2 expression if more than 10% of the cells were classified as 2 + or 3+, as described by Osman *et al.*[12] HER-2 staining was membranal in location.

Bone marrow biopsy

A bone marrow biopsy was taken from the posterior superior iliac crest which was used to prepare four "touch preps" using silanized slides (DAKO, USA). The slides were air-dried for 24 h and fixed in a solution of 70% ethanol, 5% formaldehyde, and 25% PBS for 5 min and then washed three times with PBS.

Prostate cells were detected using a monoclonal antibody directed against PSA, clone 28A4 (Novocastra Laboratories, UK), and identified using an alkaline phosphatase – anti-alkaline phosphatase-based system (LSAB2, DAKO, USA), with new fuchsin serving as the chromogen. Positive samples were divided into equal halves and were further analyzed for the expression of MMP-2 and HER-2, as previously described. The expression of MMP-2 was further characterized as being central in location or located at the edge of the micrometastasis [Figure 2].

Statistical analysis

Descriptive statistics were used for demographic variables, expressed as mean and standard deviation in case of continuous variables with a normal distribution. In case of an asymmetrical distribution, the median and interquartile range values were used. Noncontiguous variables were presented as frequencies. The Shapiro–Wilk test was used to determine a normal distribution. The Student's *t*-test was used to compare continuous variables with a normal distribution, the Mann–Whitney test for ordinate and continuous variables with a nonnormal distribution, and Chi-squared test for the differences in frequencies. Statistical significance was defined as $P < 0.05$, and all tests were two-sided. Area under the curve analysis was performed using the online program Vassarcalc (Vassarstats.net).

RESULTS

A total of 338 men who underwent radical prostatectomy participated in the study, and among them, 36.6% (120) were ≥ 70 years (older men) while the remaining 63.4% (218) were < 70 years (younger men). The clinicopathological characteristics of the two age groups are shown in Table 1. There were no significant differences between the two groups with regards to the clinicopathological findings.

Baseline characteristics of circulating prostate cells and micrometastasis 3 months postsurgery

There was no significant difference between the age groups in the frequency of men with secondary CPCs and/or micrometastasis.

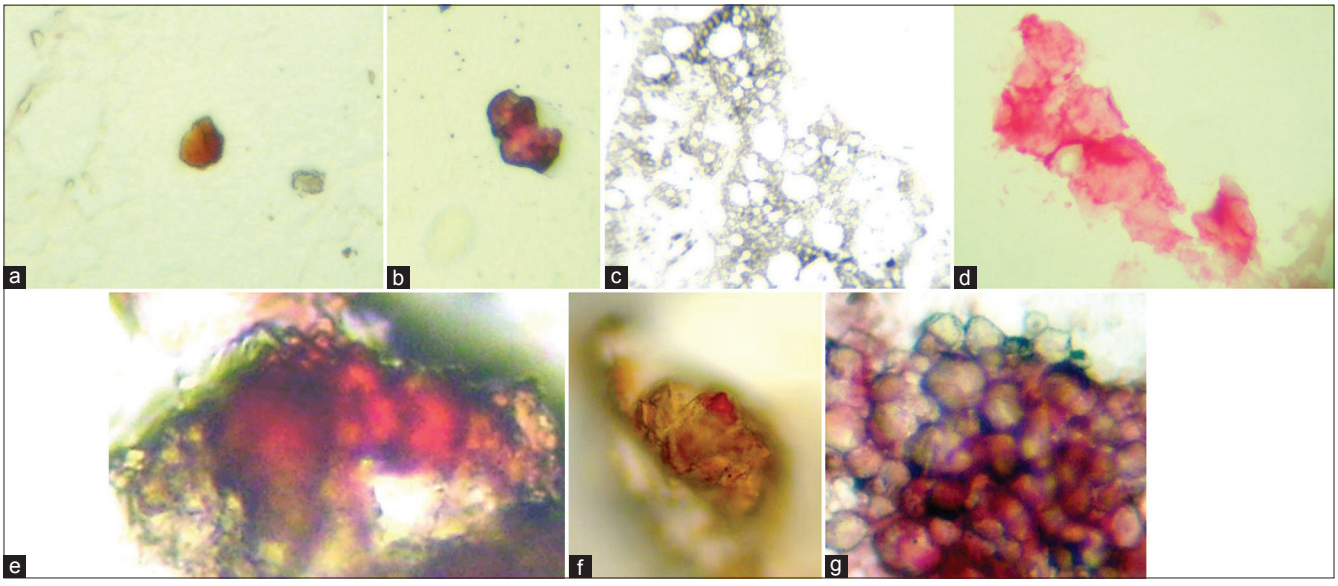

Figure 2. (a) Circulating prostate cell prostate-specific antigen(+) (red) matrix metalloproteinase 2(+) (brown), (b) circulating prostate cell prostate-specific antigen(+) (red), HER-2 (3+) (brown), (c) micrometastasis negative, (d) micrometastasis positive (prostate-specific antigen - red), (e) micrometastasis HER-2(+), (f) micrometastasis HER-2 negative, (g) micrometastasis matrix metalloproteinase 2 positive (red with brown rim), stromal cells matrix metalloproteinase 2(+) (clear with brown rim)

However, men ≥ 70 years exhibited a higher frequency of HER-2 positivity in both CPCs ($P < 0.013$) and micrometastasis ($P < 0.04$) and a higher frequency of MMP-2 positivity ($P < 0.01$) in micrometastasis, compared to younger men. Patients expressing both HER-2 and MMP-2 were more frequently found in older men ($P < 0.002$) [Table 2].

Biochemical failure

There was no significant difference in the frequency of biochemical failure between the age groups: 33.0% (72/218) in younger men *vs.* 41.7% (50/120) in older men ($P = 0.11$). In addition, there was no significant difference in the meantime to biochemical failure: 3.29 ± 2.80 years in younger men *vs.* 4.08 ± 2.91 years in older men ($P = 0.28$). In both groups, the frequency of biochemical failure was associated with the known pathological risk factor – the presence of secondary CPCs and micrometastasis [Table 3]. Among the groups, pT3 disease, Gleason score ≥ 8, extracapsular extension, and presence of secondary CPCs found significantly more frequently in older men [Table 3].

The association between the presence of secondary circulating prostate cells and pathological findings

Secondary CPCs were detected in 35.8% (78/218) of younger men and 40.0% (48/120) of older men ($P = 0.44$). In both groups, the frequency of secondary CPC detection increased with increasing pathological stage, higher Gleason score, and positive surgical margins. In older men, there was a significant association between the frequency of secondary CPC detection and extracapsular extension ($P < 0.001$), which was not seen in younger men ($P = 0.44$). Overall, the frequency of the secondary CPCs detection was significantly higher in the older men with pathological features of pT3 disease, Gleason score ≥ 8, and the positive extracapsular extension when compared to the younger men with the same pathological features [Table 4].

Phenotypic characteristics of secondary circulating prostate cells and micrometastasis and biochemical failure

In the younger group, 58/78 (74.4%) of CPC-positive patients and 50/82 (61.0%) of micrometastasis-positive patients demonstrated biochemical failure. In the older group, 44/48 (91.7%) of CPC-positive and 40/58 (69.0%) of micrometastasis-positive patients demonstrated biochemical failure. There was no statistically significant difference between the groups in the frequency of biochemical failure ($P = 0.06$ for men positive for CPCs and $P = 0.33$ for micrometastasis).

The frequency of cell expression of HER-2 and MMP-2 of secondary CPCs and micrometastasis in patients who underwent biochemical failure is shown in Table 5. The frequency of detection of HER-2 positive in the secondary CPCs and micrometastasis was significantly higher in men aged ≥ 70 years ($P = 0.013$ and $P = 0.04$, respectively). However, the rate of HER-2 expression between CPCs and micrometastasis did not show a significant difference. All CPCs expressed MMP-2 with no significant difference in their expression level among the groups. In micrometastasis, the expression of MMP-2 was found in cells at the edge of the bone fragment (peripheral distribution), which was significantly higher in older men ($P = 0.01$) than younger men. The frequency of HER-2(+) and MMP-2(+) micrometastasis was significantly higher in older men ($P = 0.002$) [Table 5].

Table 1. Clinicopathological characteristics of the two populations

	< 70 years old (n = 218) (%)	≥ 70 years old (n = 120) (%)	P
Age (mean ± SD, year)	61.1 ± 6.4	74.2 ± 3.2	
PSA (ng/mL)			
Mean	6.89	8.43	
Median (IQR)	5.39 (4.61–7.72)	5.81 (4.95–9.00)	0.113
Pathological stage			
T1	82 (37.6)	32 (26.7)	
pT2	96 (44.0)	66 (55.0)	
pT3	40 (18.4)	22 (18.3)	0.082
Gleason score			
≤ 6	154 (70.6)	86 (71.7)	
7	40 (18.4)	20 (16.7)	
≥ 8	24 (11.0)	14 (11.6)	0.691
Extracapsular extension	82 (37.6)	46 (38.0)	0.894
Positive surgical margins	32 (14.7)	20 (16.7)	0.630
Seminal vesicle (+)	4 (1.8)	4 (3.3)	0.622
Lymph node (+)	2 (0.9)	2 (1.6)	0.923

MMP-2: Matrix metalloproteinase 2; HER-2: Human epidermal growth factor receptor 2; SD: Standard deviation; IQR: Interquartile range

Table 2. Presence and phenotypic characteristics of circulating prostate cells and micrometastasis at baseline

	< 70 years old (n = 218) (%)	≥ 70 years old (n = 120) (%)	P
CPC positive	72 (35.8)	48 (40.0)	0.440
CPCs MMP-2 (+)	72 (100)	48 (100)	1.000
CPCs HER-2 (+)	17 (23.6)	23 (47.9)	0.013
Micrometastasis positive	82 (37.6)	58 (48.3)	0.054
Micrometastasis MMP-2 (+)	13 (15.9)	23 (39.7)	0.010
Micrometastasis HER-2 (+)	20 (24.4)	26 (44.8)	0.040
HER-2 (+) MMP-2 (+)	4 (4.9)	16 (27.6)	0.002

MMP-2: Matrix metalloproteinase 2; HER-2: Human epidermal growth factor receptor 2; CPCs: Circulating prostate cells

Effect of androgen suppression treatment on micrometastasis

All micrometastasis-positive patients, 49 younger men and 40 older men, underwent androgen blockade. At the beginning of the treatment, all of them showed a decrease of serum PSA levels. After 2 years of treatment, 21/49 (42.9%) of younger

Table 3. Pathological features of the two groups representing biochemical failure

	< 70 years old	≥ 70 years old	*P*
Gleason score			
≤ 6	34/154 (22.1)	22/86 (25.6)	0.602
7	24/40 (60.0)	14/20 (70.0)	0.453
≥ 8	14/24 (58.3)	14/14 (100)	0.021
P	< 0.001	< 0.001	
Pathological stage			
pT1	6/82 (7.3)	6/32 (18.8)	0.151
pT2	40/96 (41.7)	24/66 (36.4)	0.602
pT3	26/40 (65.0)	20/22 (91.0)	0.030
P	< 0.001	< 0.001	
Extracapsular extension			
Positive	46/82 (56.1)	36/46 (78.3)	
Negative	50/186 (26.9)	14/74 (18.9)	0.021
P	< 0.001	< 0.001	
Surgical margins			
Positive	22/32 (68.8)	16/20 (80.0)	0.572
Negative	50/186 (26.9)	34/100 (34)	
P	< 0.001	< 0.001	
CPCs			
Positive	58/78 (74.4)	44/48 (91.7)	0.021
Negative	20/140 (14.3)	6/72 (8.3)	
P	< 0.001	< 0.001	
Micrometastasis			
Positive	50/82 (61.0)	40/58 (69.0)	0.334
Negative	22/136 (16.2)	10/62 (16.1)	
P	< 0.001	< 0.001	

MMP-2: Matrix metalloproteinase 2; HER-2: Human epidermal growth factor receptor 2; CPCs: Circulating prostate cells

men showed sustained increase in serum PSA compared to 33/40 (82.5%) of older men ($P < 0.001$, relative risk [RR] 1.93 [95% confidence interval (CI) 1.35–2.74], odds ratio [OR] 6.29 [95% CI 2.34–16.96]). Repeat bone marrow biopsy in younger men showed 24/49 (49.0%) to be micrometastasis negative *vs.* 6/40 (15.0%) in older men ($P < 0.001$, RR 1.67 [95% CI 1.23–2.26], OR 5.44 [95% CI 1.93–15.28]). More younger men had significantly become micrometastasis negative after 2 years of androgen blockade treatment and maintained a low serum PSA level ($P < 0.001$).

None of the men with HER-2-positive micrometastasis before androgen blockade became micrometastasis negative, irrespective of their age. After 2 years of androgen blockade, there was no difference in the frequency of HER-2 expression in the micrometastasis between the age groups; however, MMP-2 expression was higher in older patients ($P = 0.032$) [Table 6].

Table 4. Association of the presence or absence of secondary circulating prostate cells with pathological findings and biochemical failure

	< 70 years old (n = 78) (%)	≥ 70 years old (n = 48) (%)	*P*
Pathological stage			
pT1	11/82 (13.4)	7/32 (21.9)	0.275
pT2	42/96 (43.8)	22/66 (33.3)	0.189
pT3	25/40 (62.5)	19/22 (86.4)	
P	< 0.001	< 0.001	0.047
Gleason score			
≤ 6	34/154 (22.1)	22/88 (25.0)	0.603
7	30/40 (75.0)	13/20 (65.0)	0.425
≥ 8	14/24 (58.3)	13/14 (92.9)	0.024
P	< 0.001	< 0.001	
Extracapsular extension			
Positive	32/82 (39.0)	32/46 (69.6)	0.009
Negative	46/136 (33.8)	16/74 (21.6)	0.064
P	0.44	> 0.001	
Surgical margins			
Positive	22/32 (68.8)	17/20 (85.0)	0.193
Negative	56/186 (30.1)	31/100 (31.0)	0.898
P	< 0.001	< 0.001	

MMP-2: Matrix metalloproteinase 2; HER-2: Human epidermal growth factor receptor 2

DISCUSSION

Results from studies on the effect of age on the risk of biochemical failure are conflicting.[2-4] In our study, in a group of 328 men who underwent radical prostatectomy, there were no significant differences with respect to known risk factors for biochemical failure. In addition, there was no difference in the frequency of biochemical failure or the meantime to become biochemical failure between the two groups, which supports the results of previous studies.[4,5] However, subgroup analysis showed that elderly men with Gleason score ≥ 8, pT3 tumors, positive extracapsular extension, and presence of secondary CPCs, but not positive surgical margins or bone marrow micrometastasis, had a significantly higher frequency of biochemical failure.

The higher frequency of secondary CPCs in older men with a Gleason score ≥ 8, pT3 disease, or extracapsular extension suggests that they may represent a more aggressive form of the disease with active dissemination of tumor cells, either from bone marrow micrometastasis or locally from the prostate bed and surrounding tissues.

Our study showed that secondary CPCs and bone marrow micrometastasis in elderly men had a significantly higher frequency of HER-2 expression. It has been reported that HER-2-positive cells in the original tumor have an increased

Table 5. Expression of human epidermal growth factor receptor 2 and matrix metalloproteinase 2 in circulating prostate cells and micrometastasis

	< 70 years old (n = 78) (%)	≥ 70 years old (n = 48) (%)	P
CPCs			
N° positive	58 (74.4)	44 (91.7)	
HER-2(+)	14 (24.1)	21 (47.7)	0.013
MMP-2(+)	58 (100)	44 (100)	1.000
Micrometastasis			
N° positive	50 (64.1)	40 (83.3)	
HER-2(+)	12 (24.0)	18 (45.0)	0.040
MMP-2(+)	7 (14.0)	15 (37.5)	0.010
HER-2(+) MMP-2(+)	2 (4.0)	11 (27.5)	0.002

MMP-2: Matrix metalloproteinase 2; HER-2: Human epidermal growth factor receptor 2; CPCs: Circulating prostate cells

Table 6. Expression of human epidermal growth factor receptor 2 and matrix metalloproteinase 2 in micrometastasis of men 2 years after androgen blockade

	< 70 years old (n = 49) (%)	≥ 70 years old (n = 40) (%)	P
Micrometastasis			
N° positive	26 (53.1)	34 (85.0)	0.003
HER-2(+)	17 (65.4)	29 (85.3)	0.130
MMP-2(+)	12 (46.2)	26 (76.5)	0.032
HER-2(+) MMP-2(+)	12 (46.2)	24 (70.6)	0.090

MMP-2: Matrix metalloproteinase 2; HER-2: Human epidermal growth factor receptor 2

ability to infiltrate blood vessels and thus escape into the circulation.[29] These cells having disseminated before surgery are not affected by local treatment, may implant in distant tissues, and are a cause of later treatment failure. This may explain the significant difference in biochemical failure in older patients with extracapsular extension but with negative margins. This difference in the phenotypic characteristics of CPCs may explain the results of Masuda *et al.*,[30] who reported that older men with negative margins and pT2 disease had a significantly higher frequency of biochemical failure than younger men with the same characteristics.

The phenotypic characteristics of CPCs and micrometastasis did not significantly change between the initial sample and biochemical failure sample. This suggests that in the presence of androgens, there was no selective advantage of expressing HER-2 or MMP-2. The presence of CPCs was associated with known pathological risk factors for biochemical failure and is consistent that they represent a more aggressive disease.

In the presence of androgen blockade, there was a significant change in HER-2 expression in both groups. Posttreatment, the frequency of HER-2 expression was significantly higher compared to pretreatment frequencies. The fact that the serum PSA decreased with the start of androgen blockade is consistent with the theory that the sensitive HER-2 negative cells are eliminated, leaving the resistant HER-2-positive cells to proliferate and grow later. The higher expression of HER-2 in older men would explain why more of these patients progressed to castrate resistant cancer and inversely why fewer had eradication of micrometastasis; this has clinical implications. Pantel *et al.*[31] reported that after neoadjuvant androgen blockade, 16/21 (76.2%) of patients became negative for bone marrow micrometastasis while 4/21 (19.0%) had a decreased number of tumor cells detected. Köllermann *et al.*[32] reported that patients who remained positive for bone marrow micrometastasis demonstrated worse prognosis after neoadjuvant androgen blockade. These two studies, together with our data, suggest that although it is possible to eradicate bone marrow micrometastasis, patients who remain positive will have a worse prognosis. In patients with HER-2-positive micrometastasis and CPCs, androgen blockade seems to be much less effective and in such patients, alternative treatment may be more appropriate. It also raises the questions that if HER-2-negative bone marrow micrometastasis can be eliminated by androgen blockade and whether its early use in these patients could lead to a better survival.

Why elderly men express a higher frequency of HER-2 expression is unknown. One possible explanation is that elderly men tend to have lower levels of testosterone,[33] which has been associated with a more aggressive disease[34] and an increased risk of biochemical failure.[35] In men with lower testosterone levels, there could be selection of HER-2-positive cells, explaining the higher frequency of detection in older men. These HER-2-positive cells have an increased ability for vascular dissemination, possibly via MMP-2 stimulation,[21,36] and thus escape the effects of local treatment. When implanted in the bone marrow, it results in a higher frequency of MMP-2 expression, suggesting that these cells continue with the ability to redisseminate. This increased expression of MMP-2 facilitates dissemination of tumor cells from the micrometastasis into the circulation because of its gelatinase activity on the extracellular matrix.

In conclusion, elderly men appear to have a more aggressive phenotype of prostate cancer which in clinical practice is reflected as a higher frequency of occurrence of disease which is resistant to androgen blockade. The phenotypic characteristics of bone marrow micrometastasis and CPCs may guide the choice of treatment in these patients.

Acknowledgments

We wish to thank Mrs. Ana Maria Palazuelos for her help in the writing of this manuscript.

Financial support and sponsorship

The study was funded by a Servicio de Salud Metropolitano Oriente research grant 2008.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. 2015 NCCN Clinical Practice Guidelines in Oncology on Prostate Cancer. Available from: https://www.nccn.org/professionals/physician-gls/pdf/prostate.pdf. [Last accessed on 2015 Aug 05].
2. Brassell SA, Rice KR, Parker PM, Chen Y, Farrell JS, Cullen J, McLeod DG. Prostate cancer in men 70 years old or older, indolent or aggressive: clinicopathological analysis and outcomes. *J Urol* 2011; 185 (1): 132–7.
3. Kim JK, Cho SY, Jeong CW, Lee SB, Ku JH, Hong SK, Byun SS, Kwak C, Him HH, Lee SE, Jeong H. Patients aged more than 70 had higher risk of locally advanced prostate cancers and biochemical recurrence in Korea. *BJU Int* 2012; 110 (4): 505–9.
4. Malaeb BS, Rashid HH, Lotan Y, Khoddami SM, Shariat SF, Sagalowsky AI, McConnell JD, Roehrborn CG, Koeneman KS. Prostate cancer disease-free survival after radical retropubic prostatectomy in patients older than 70 years compared to younger cohorts. *Urol Oncol* 2007; 25 (4): 291–7.
5. Magheli A, Rais-Bahrami S, Humphreys EB, Peck HJ, Trock BJ, Gonzalgo ML. Impact of patient age on biochemical recurrence rates following radical prostatectomy. *J Urol* 2007; 178 (5): 1933–7.
6. Murray NP, Reyes E, Orellana N, Fuentealba C, Dueñas R. Elimination of primary circulating prostate cells after radical prostatectomy for prostate cancer decreases the risk of future biochemical failure. *Arch Esp Urol* 2014; 67 (8): 684–91.
7. Murray NP, Reyes E, Orellana N, Fuentealba C, Bádinez L, Olivares R, Porcell J, Dueñas R. Secondary circulating prostate cells predict biochemical failure in prostate cancer patients after radical prostatectomy and without evidence of disease. *Scientific World Journal* 2013. doi: 10.1155/2013/762064.
8. Wood DP Jr., Banerjee M. Presence of circulating prostate cells in the bone marrow of patients undergoing radical prostatectomy is predictive of disease-free survival. *J Clin Oncol* 1997; 15 (12): 3451–7.
9. Murray NP, Reyes E, Tapia P, Badinez L, Orellana N, Fuentealba C, Olivares R, Porcell J, Dueñas R. Redefining micrometastasis in prostate cancer – A comparison of circulating prostate cells, bone marrow disseminated tumor cells and micrometastasis: implications in determining local or systemic treatment for biochemical failure after radical prostatectomy. *Int J Mol Med* 2012; 30 (4): 896–904.
10. Brodsky AS, Fischer A, Miller DH, Vang S, MacLaughlan S, Wu HT, Yu J, Steinhoff M, Collins C, Smith PJ, Raphael BJ, Brard L. Expression profiling of primary and metastatic ovarian tumors reveals differences indicative of aggressive disease. *PLoS One* 2014; 9 (4): e94476.
11. Braun S, Schlimok G, Heumos I, Schaller G, Riethdorf L, Riethmüller G, Pantel K. ErbB2 overexpression on occult metastatic cells in bone marrow predicts poor clinical outcome of stage I-III breast cancer patients. *Cancer Res* 2001; 61 (5): 1890–5.
12. Osman I, Scher HI, Drobnjak M, Verbel D, Morris M, Agus D, Ross JS, Cordon-Cardo C. HER-2/neu (p185neu) protein expression in the natural or treated history of prostate cancer. *Clin Cancer Res* 2001; 7 (9): 2643–7.
13. Murray NP, Badinez LV, Dueñas RR, Orellana N, Tapia P. Positive HER-2 protein expression in circulating prostate cells and micro-metastasis, resistant to androgen blockage but not diethylstilbestrol. *Indian J Urol* 2011; 27 (2): 200–7.
14. Trudel D, Fradet Y, Meyer F, Harel F, Têtu B. Significance of MMP-2 expression in prostate cancer: an immunohistochemical study. *Cancer Res* 2003; 63 (23): 8511–5.
15. Signoretti S, Montironi R, Manola J, Altimari A, Tam C, Bubley G, Balk S, Thomas G, Kaplan I, Hlatky L, Hahnfeldt P, Kantoff P, Loda M. Her-2-neu expression and progression toward androgen independence in human prostate cancer. *J Natl Cancer Inst* 2000; 92 (23): 1918–25.
16. Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nat Med* 1999; 5 (3): 280–5.
17. Shi Y, Chatterjee SJ, Brands FH, Shi SR, Pootrakul L, Taylor CR, Datar R, Cote RJ. Role of coordinated molecular alterations in the development of androgen-independent prostate cancer: an *in vitro* model that corroborates clinical observations. *BJU Int* 2006; 97 (1): 170–8.
18. D'Antonio JM, Ma C, Monzon FA, Pflug BR. Longitudinal analysis of androgen deprivation of prostate cancer cells identifies pathways to androgen independence. *Prostate* 2008; 68 (7): 698–714.
19. Wen Y, Hu MC, Makino K, Spohn B, Bartholomeusz G, Yan DH, Hung MC. HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. *Cancer Res* 2000; 60 (24): 6841–5.
20. Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C. From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. *Proc Natl Acad Sci U S A* 1999; 96 (10): 5458–63.
21. Murray NP, Reyes E, Badinez L, Orellana N, Fuentealba C, Olivares R, Porcell J, Dueñas R. Effect of androgen blockade on HER-2 and matrix metalloproteinase-2 expression on bone marrow micrometastasis and stromal cells in men with prostate cancer. *Scientific World Journal* 2013. doi: 10.1155/2013/281291.
22. Ke Z, Lin H, Fan Z, Cai TQ, Kaplan RA, Ma C, Bower KA, Shi X, Luo J. MMP-2 mediates ethanol-induced invasion of mammary epithelial cells over-expressing ErbB2. *Int J Cancer* 2006; 119 (1): 8–16.
23. Bao W, Fu HJ, Jia LT, Zhang Y, Li W, Jin BQ, Yao LB, Chen SY, Yang AG. HER2-mediated upregulation of MMP-1 is involved in gastric cancer cell invasion. *Arch Biochem Biophys* 2010; 499 (1-2): 49–55.
24. Trudel D, Fradet Y, Meyer F, Harel F, Têtu B. Membrane-type-1 matrix metalloproteinase, matrix metalloproteinase 2, and tissue inhibitor of matrix proteinase 2 in prostate cancer: identification of patients with poor prognosis by immunohistochemistry. *Hum Pathol* 2008; 39 (5): 731–9.
25. Zhong WD, Han ZD, He HC, Bi XC, Dai QS, Zhu G, Ye YK, Liang YX, Qin WJ, Zhang Z, Zeng GH, Chen ZN. CD147, MMP-1, MMP-2 and MMP-9 protein expression as significant prognostic factors in human prostate cancer. *Oncology* 2008; 75 (3-4): 230–6.
26. Stearns M, Stearns ME. Evidence for increased activated metalloproteinase 2 (MMP-2a) expression associated with human prostate cancer progression. *Oncol Res* 1996; 8 (2): 69–75.
27. Xiao LJ, Lin P, Lin F, Liu X, Qin W, Zou HF, Guo L, Liu W, Wang SJ, Yu XG. ADAM17 targets MMP-2 and MMP-9 via EGFR-MEK-ERK pathway activation to promote prostate cancer cell invasion. *Int J Oncol* 2012; 40 (5): 1714–24.
28. Littlepage LE, Sternlicht MD, Rougier N, Phillips J, Gallo E, Yu Y, Williams K, Brenot A, Gordon JI, Werb Z. Matrix metalloproteinases contribute distinct roles in neuroendocrine prostate carcinogenesis, metastasis, and angiogenesis progression. *Cancer Res* 2010; 70 (6): 2224–34.
29. Murray NP, Reyes E, Fuentealba C, Jacob O, Orellana N. Possible role of HER-2 in the Progression of prostate cancer from primary tumor to androgen independence. *Asian Pac J Cancer Prev* 2015; 16 (15): 6615–9.
30. Masuda H, Fukushima H, Kawakami S, Numao N, Fujii Y, Saito K, Koga F, Ishioka J, Yokoyama M, Kihara K. Impact of advanced age on biochemical recurrence after radical prostatectomy in Japanese men according to pathological stage. *Jpn J Clin Oncol* 2013; 43 (4): 410–6.
31. Pantel K, Enzmann T, Köllermann J, Caprano J, Riethmüller G, Köllermann MW. Immunocytochemical monitoring of micrometastatic disease: reduction of prostate cancer cells in bone marrow by androgen deprivation. *Int J Cancer* 1997; 71 (4): 521–5.
32. Köllermann J, Weikert S, Schostak M, Kempkensteffen C, Kleinschmidt K, Rau T, Pantel K. Prognostic significance of disseminated tumor cells in the bone marrow of prostate cancer patients treated with neoadjuvant hormone treatment. *J Clin Oncol* 2008; 26 (30): 4928–33.
33. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR; Baltimore Longitudinal Study of Aging. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab* 2001; 86 (2): 724–31.
34. Xylinas E, Ploussard G, Durand X, Fabre A, Salomon L, Allory Y, Vordos D, Hoznek A, Abbou CC, de la Taille A. Low pretreatment

total testosterone (<3 ng/mL) predicts extraprostatic disease in prostatectomy specimens from patients with preoperative localized prostate cancer. *BJU Int* 2011; 107 (9): 1400–3.
35. Yamamoto S, Yonese J, Kawakami S, Ohkubo Y, Tatokoro M, Komai Y, Takeshita H, Ishikawa Y, Fukui I. Preoperative serum testosterone level as an independent predictor of treatment failure following radical prostatectomy. *Eur Urol* 2007; 52 (3): 696–701.
36. Liao X, Thrasher JB, Pelling J, Holzbeierlein J, Sang QX, Li B. Androgen stimulates matrix metalloproteinase-2 expression in human prostate cancer. *Endocrinology* 2003; 144 (5): 1656–63.