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Outcome after BCG treatment for urinary bladder cancer may be influenced by polymorphisms in the *NOS2* and *NOS3* genes ☆

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ABSTRACT

Purpose: Bacillus Calmette-Guérin (BCG)-treatment is an established treatment for bladder cancer, but its mechanisms of action are not fully understood. High-risk non-muscle invasive bladder-cancer (NMIBC)-patients failing to respond to BCG-treatment have worse prognosis than those undergoing immediate radical cystectomy and identification of patients at risk for BCG-failure is of high priority. Several studies indicate a role for nitric oxide (NO) in the cytotoxic effect that BCG exerts on bladder cancer cells. In this study we investigated whether *NO-synthase (NOS)*-gene polymorphisms, *NOS2*-promoter microsatellite (CCTTT)n, and the *NOS3*-polymorphisms-786T > C (rs2070744) and Glu298Asp (rs1799983), can serve as possible molecular markers for outcome after BCG-treatment for NMIBC.
Materials and methods: All NMIBC-patients from a well-characterized population based cohort were analyzed ($n=88$). Polymorphism data were combined with information from 15-years of clinical follow-up. The effect of BCG-treatment on cancer-specific death (CSD), recurrence and progression in patients with varying *NOS*-genotypes were studied using Cox proportional hazard-models and log rank tests.
Results: BCG-treatment resulted in significantly better survival in patients without (Log rank: $p=0.006$; HR: 0.12, $p=0.048$), but not in patients with a long version ((CCTTT)n ≧13 repeats) of the *NOS2*-promoter microsatellite. The *NOS3*-rs2070744(TT) and rs1799983(GG)-genotypes showed decreased risk for CSD (Log rank(TT): $p=0.001$; Log rank(GG): $p=0.010$, HR(GG): 0.16, $p=0.030$) and progression (Log rank(TT): $p<0.001$, HR(TT): 0.05, $p=0.005$; Log rank(GG): $p<0.001$, HR(GG): 0.10, $p=0.003$) after BCG-therapy compared to the other genotypes. There was also a reduction in recurrence in BCG-treated patients that was mostly genotype independent. Analysis of combined genotypes identified a subgroup of 30% of the BCG-treated patients that did not benefit from BCG-treatment.
Conclusions: Our results suggest that the investigated polymorphisms influence patient response to BCG-treatment and thus may serve as possible markers for identification of BCG-failures.

1. Introduction

Urinary bladder cancer accounts for up to 5% of all new cancers in the Western world, and commonly presents as a highly differentiated tumor confined to the bladder mucosa (Ta) or sub-mucosa (T1) [1]. Despite advances in management of non-muscle invasive bladder cancer (NMIBC), tumor recurrence and progression rates remain high.

Treatment with Bacillus Calmette-Guérin (BCG) is considered the most effective intravesical treatment for NMIBC. NMIBC can be divided into low, intermediate and high-risk bladder cancer with respect to recurrence and disease progression [1]. In general, BCG is not advocated for low-risk NMIBC due to its favorable prognosis. In intermediate risk NMIBC, BCG is a well-established treatment

Abbreviations: BCG, Bacillus Calmette-Guérin vaccine; NMIBC, non-muscle invasive bladder cancer; NOS, nitric oxide synthase; CSD, cancer specific death; CI, confidence interval

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for disease recurrence but its role in preventing progression is controversial. In high-risk NMIBC, e.g. high grade (G3) and stage T1 tumors, BCG has been shown to reduce disease progression [2], and is still the most frequently used bladder-sparing option available. Despite its efficacy, a significant proportion of BCG-treated patients (30–35%) either relapse within the first five years after treatment or fail to respond at all [3]. High-risk NMIBC-patients failing to respond to BCG-treatment, and undergoing radical cystectomy as second treatment , generally have a worse prognosis than those undergoing immediate radical cystectomy [4]. Thus, identification of patients at risk for BCG failure is of high priority.

Although BCG is an established treatment for bladder cancer its precise mechanisms of action are not fully understood. Several studies indicate a role for nitric oxide (NO) in the cytotoxic effect that BCG exerts on bladder cancer cells [5,6]. NO is a biological messenger with the ability to induce both anti-microbial and anti-tumoral effects depending on cellular context. It is synthesized from the conversion of L-arginine to L-citrullin by a family of three NO synthases (*NOS1-3*). Altered expression of *NOS2* and *NOS3* has been described in several human cancer forms, among them bladder cancer, implicating that they may take part in cancer biology [7–12]. Previously, we found associations between the *NOS2* promoter microsatellite (CCTTT)n polymorphism, the *NOS3* promoter polymorphism rs2070744 (-786T > C), the *NOS3* polymorphism rs1799983 (Glu298Asp) and the development and progression of bladder cancer [8,12].

To evaluate polymorphisms as biological markers for bladder cancer progression and patient survival, we here investigate the association between *NOS2* and *NOS3* polymorphism in high-risk NMIBC patients with respect to patient outcome after BCG-treatment.

2. Materials and methods

2.1. Study population

The patients included in this prospective study were drawn from a population-based bladder cancer cohort consisting of 78% (563/721) of all newly diagnosed bladder-cancer patients in the Stockholm county, Sweden during 1995–1996, as previously described [13]. For these patients we now have 15 years clinical follow up, defined as the time between diagnosis and last clinical evaluation or death. Parameters registered were date of diagnosed tumor recurrences, progress in grade/stage, development of nodal or distant metastases, type of therapy and cause of death. For grading the WHO1999 malignancy-grading system [14] was used and tumor stage was assessed according to a modified TNM system suggested by Hall and Prout [15]. Venous blood (normal tissue) was collected at a later time point and was available for 359 patients, who were genotyped for the *NOS2* promoter microsatellite (CCTTT)n polymorphism, the *NOS3* promoter polymorphism rs2070744 (-786T > C), and the *NOS3* exon 7 polymorphism rs1799983 (Glu298Asp) [8,12]. For the present study, all patients with high-risk NMIBC (i.e. either TaG3, T1, TaG1 + concomitant carcinoma in situ (conCIS), TaG2 + conCIS or primary CIS transitional cell carcinoma), i.e. 25% of the patients (88/359), were included. Genotyping data for the *NOS2* and *NOS3* polymorphisms were combined with clinical parameters on cancer-specific death (CSD), disease progression and recurrence. Informed written consent was obtained from all participants, and the study was approved by the regional Ethical Committee.

2.2. Study design

The NOS2 (CCTTT)n microsatellite promoter (−2.6 kb) polymorphism was analyzed by DNA fragment analysis as previously described [8]. The *NOS3* polymorphisms rs2070744 and rs1799983 were analyzed by Allelic discrimination on an ABI Prism® 7900HT sequence detection system, also as previously described [12]. The researcher performing the genotyping did not have any pre-knowledge of what treatment the different patients had received. Based on our previous observation that patients homozygous for a long set of repeats of the (CCTTT)n microsatellite had a higher risk for stage progression and CSD [8], we grouped the patients into carriers of a long allele (L-carrier, (CCTTT)n ≥ 13 repeats) or non-carriers (non-L-carrier), with the hypothesis that carrying long repeats was a disadvantage. For the *NOS3* polymorphisms, recessive models were used for the statistical analyses due to the low frequencies of individuals homozygous for the minor *NOS3*-alleles. Thus, for the *NOS3* rs2070744 polymorphism individuals homozygous for the minor C-allele (n=8/87; 5.7%) were analyzed together with the heterozygotes, as a CT/CC-group. For the *NOS3* rs1799983 polymorphism the patients homozygous for the minor T-allele (n=4/64; 2.9%) were analyzed together with the heterozygotes, as a GT/TT-group.

2.3. Statistical analysis

Age, sex and tumor stage and grade adjusted hazard ratios (HR) were calculated with stepwise backward selection models (Cox proportional hazards) to assess the risk of CSD (defined as bladder cancer as the cause of death according to medical records), disease progression (defined as tumor stage T2 or more advanced, the presence of a metastasis (nodal or distant), or death caused by bladder cancer) and recurrence (defined as return of disease in any form; In the group of BCG-treated patients, recurrence that occurred before BCG treatment started has been excluded from the study) in BCG responders and non-responders. All HRs are shown with a 2-sided 95% Confidence Interval (CI). Follow-up was defined as time from date of diagnosis to last clinical evaluation or death from bladder cancer or other causes. Follow-up time for progression was defined as time from date of diagnosis to first progression event, death or last clinical evaluation. Kaplan–Meier estimator plot with the 2-sided log rank test is provided to visualize the cumulative effect of polymorphisms over time. Associations between the polymorphisms were calculated with linkage disequilibrium analyses and Pearson's coefficient of correlation. Statistical analyses were done with IBM® SPSS® Statistics, version 21.0.

3. Results

The characterization of the study population is described in Table 1. Overall, we found a significant reduction in recurrence for those patients who had received BCG. This reduction was independent of genotype, with the exception of the rs1799983 polymorphism, where the BCG-treated patients homozygous for the G allele (GG) had a decreased risk for recurrence.

3.1. NOS2(CCTTT)n microsatellite promoter (−2.6 kb) polymorphism

Genotyping of the (CCTTT)n microsatellite promoter polymorphism was successful in all 88 patients with high-risk NMIBC. Of these, 51 patients had received BCG-treatment at some point. In L-carrier patients (n=45) there were no differences in CSD between those who had received BCG and those who had not (Table 2). However, in non-L-carriers (n=43) the risk of CSD was significantly reduced for those patients who had received BCG (HR: 0.12; CI: 0.02–0.98; p=0.048) (Table 2). There was also a reduction in disease progression for those who had received BCG,

Table 1
Study population.

	BCG treated		Non-BCG treated		Total	
	N.o.Pts (%)[a]	conCIS[b]	N.o.Pts (%)[a]	conCIS[b]	N.o.Pts (%)[a]	conCIS[b]
TaG3	9 (18)	4	4 (11)	0	13 (15)	4
T1G1/G2	11 (22)	0	18 (49)	0	29 (33)	0
T1G3	19 (37)	5	15 (41)	2	34 (39)	7
TaG1/G2+conCIS	3 (6)	3	0 (0)	0	3 (3)	3
Primary CIS	9 (18)	N.A.	0 (0)	N.A.	9 (10)	N.A.
Total (N.o. Pts)	51		37		88	
Mean age (years)	69.7		70.7		70.4	

[a] Number (N.o. Pts) and percentage (%) of high-risk NMIBC-patients with different tumor stage and grade.
[b] Number of patients within each tumor stage and grade category having concomitant carcinoma in situ (conCIS).

but this was not enhanced by the (CCTTT)n polymorphism, as no difference was found between the L-carrier and the non-L-carrier groups (Table 2).

3.2. *NOS3 promoter polymorphism rs2070744*

Genotyping was successful in 87/88 (99%) of the high-risk NMIBC patients of which 50 had received BCG-treatment. None of the TT-patients, 0/21 (0.0%), treated with BCG died from bladder cancer during the time of follow-up, while 7 out of the 18 (38.9%) TT-patients who were not treated with BCG died from CSD during the same period (Table 2). Also, TT-patients had a significantly decreased risk for disease progression after BCG-treatment (HR: 0.05; CI: 0.01–0.42; $p=0.005$, Table 2). In patients with the CT and CC genotypes, BCG gave no advantage regarding CSD and disease progression compared to those who had not received BCG (Table 2).

3.3. *NOS3 exon polymorphism rs1799983*

Genotyping was successful in 64/88 (76%) of the high-risk NMIBC patients, of which 34 had received BCG-treatment. Patients homozygous for the G allele (GG) and treated with BCG had a significantly decreased risk for CSD compared to those who had not received BCG (HR: 0.16; CI: 0.03–0.84; $p=0.030$, Table 2). Patients homozygous for the G allele (GG) that were treated with BCG also had a decreased risk for recurrence (HR: 0.29; CI: 0.10–00.87; $p=0.028$) and for disease progression (HR: 0.10; CI: 0.02–0.46; $p=0.003$, Table 2). In patients with the GT and TT genotypes BCG gave no advantage regarding CSD, recurrence or disease progression in comparison to those who had not received BCG (Table 2)

3.4. *Combinatorial effects of NOS-genotypes*

Linkage analyzes showed a moderate correlation between the two *NOS3*-polymorphisms (r: 0.14; $p<0.001$), but no correlations between the two *NOS3*-polymorphisms and the *NOS2* (CCTTT)n microsatellite promoter polymorphism. When combining the genotypes of the investigated polymorphisms in all high-risk NMIBC-patients, those patients with one or more of either (1) *NOS2* (CCTTT)non-L-carrier (homozygous), (2) *NOS3*-rs2070744 (TT) or (3) rs1799983 (GG) genotypes, had a significant advantage from BCG-treatment regarding both CSD (HR: 0.17; CI: 0.05–0.59; $p=0.006$, Table 3) and disease progression (HR: 0.21; CI: 0.08–0.55; $p=0.002$, Table 3), while patients without these genotypes had no significant advantage of BCG-treatment.

Analyses of genotypes only within the group of BCG-treated patients ($n=51$) show that those ($n=36$; 70.6%) with at least one of either (1) *NOS2* (CCTTT)non-L-carrier (homozygous), (2) *NOS3*-rs2070744 (TT) or (3) rs1799983 (GG) genotypes had a significantly better cancer specific survival (HR: 0.20; CI: 0.05–0.85; $p=0.029$; Log-rank test: $p=0.037$), and a decreased risk of recurrence (HR: 0.54; CI: 0.23–1.27; $p=0.160$; Log-rank test: $p=0.035$), than patients without these three genotypes (Fig. 1).

4. Discussion

Our data show that the investigated *NOS*-polymorphisms are associated with progression and CSD after BCG-treatment in patients with high-risk NMIBC. By combining the investigated genotypes, we were also able to identify a subgroup of patients who did not benefit from the BCG treatment. Patients homozygous or heterozygous for a long set of *NOS2* (CCTTT)n repeats (L-carriers) had a significantly higher risk for CSD after BCG-treatment compared to the non-L-carriers. The *NOS3*-polymorphisms influenced the risk for both CSD and disease progression after BCG-treatment, in favor for those with the rs2070744 (TT) and the rs1799983 (GG)-genotypes. The effect of BCG-treatment on bladder tumor recurrence was significant across all genotype groups, except for the rs1799983 GT/TT-group where the effect was moderate. However, after stratification into treatment groups (BCG/not BCG), a combined genotype effect was seen within the BCG-treated group, with a decreased risk of recurrence in the group of patients carrying at least one of either (1) *NOS2* (CCTTT)non-L-carrier (homozygous), (2) *NOS3*- rs2070744 (TT) or (3) rs1799983 (GG) genotypes.

Several studies suggest an anti-tumor promoting effect of NO after BCG treatment [7,16]. BCG was also one of the first compounds shown to induce NO-synthesis in activated macrophages, which in turn promoted their cytotoxic effects against tumor cells [17]. However, not all tumors respond with growth arrest and apoptosis when exposed to NO. One theory, supported by several studies, is that this effect is caused by an acquired resistance to NO, following a prolonged exposure to low NO-concentrations, which later offers a protection to higher levels [18]. In accordance, mainly *NOS2*-expressing tumors, at the first resection, have been shown to recur after BCG-treatment [16,19]. It is plausible that polymorphisms in part regulate *NOS*-expression and functional in vitro studies of the (CCTTT)n repeat polymorphism have shown that increased *NOS2* gene expression correlate to an increased number of (CCTTT)n repeats, due to a more active promoter [20,21]. It is possible that patients with longer (CCTTT)n repeats after *NOS2*-induction could produce higher NO concentrations for a longer time, which by itself can be pro-metastatic in the established tumor and also cause resistance to the high levels of NO seen after BCG-treatment. In theory, this could be one explanation to why we see a repressed response to BCG in L-carriers compared to non-L-carriers.

Table 2
Cancer specific death, disease progression and recurrence in patients with high-risk NMIBC.

		N.o. Pts/total N.o. (%)	Adjusted HR (95% CI)	*p*-Value
NOS2 (CCTTT)n microsatellite promoter (−2.6 kb)				
CSD				
L-carriers	Not BCG treated	5/14 (36)	1.0	
	BCG treated	7/31 (23)	0.61 (0.19–1.92)	0.349
Non-L-carriers	Not BCG treated	9/23 (39)	1.0	
	BCG treated	1/20 (5)	0.12 (0.02–0.98)	***0.048***
Progression				
L-carriers	Not BCG treated	9/14 (64)	1.0	
	BCG treated	8/31 (26)	0.28 (0.11–0.73)	***0.009***
Non -L-carriers	Not BCG treated	12/23 (52)	1.0	
	BCG treated	3/20 (15)	0.22 (0.06–0.77)	***0.018***
Recurrence				
L-carriers	Not BCG treated	11/14 (79)	1.0	
	BCG treated	16/31 (52)	0.31 (0.14–0.69)	***0.004***
Non-L-carriers	Not BCG treated	16/23 (70)	1.0	
	BCG treated	6/20 (30)	0.20 (0.08–0.54)	***0.001***
rs2070744 (NOS3 -786T > C)				
CSD				
TT	Not BCG treated	*7/18 (39)*	1.0	
	BCG treated	*0/21 (0)*	–	[a]
CT/CC	Not BCG treated	7/19 (37)	1.0	
	BCG treated	8/29 (28)	0.81 (0.28–2.32)	0.696
Progression				
TT	Not BCG treated	11/18 (61)		
	BCG treated	1/21 (5)	0.05 (0.01–0.42)	***0.005***
CT/CC	Not BCG treated	10/19 (53)	1.0	
	BCG treated	10/29 (35)	0.53 (0.22–1.28)	0.156
Recurrence				
TT	Not BCG treated	12/18 (67)	1.0	
	BCG treated	5/21 (24)	0.23 (0.08–0.70)	***0.009***
CT/CC	Not BCG treated	15/19 (79)	1.0	
	BCG treated	17/29 (59)	0.25 (0.11–0.54)	***< 0.001***
rs1799983 (NOS3 Glu298Asp)				
CSD				
GG	Not BCG treated	6/12 (50)	1.0	
	BCG treated	2/17 (12)	0.16 (0.03–0.84)	***0.030***
GT/TT	Not BCG treated	6/18 (33)	1.0	
	BCG treated	4/17 (24)	0.88 (0.24–3.22)	0.845
Progression				
GG	Not BCG treated	9/12 (75)	1.0	
	BCG treated	3/17 (18)	***0.10 (0.02–0.46)***	***0.003***
GT/TT	Not BCG treated	9/18 (50)	1.0	
	BCG treated	5/17 (29)	0.67 (0.21–2.15)	0.505
Recurrence				
GG	Not BCG treated	9/12 (75)	1.0	
	BCG treated	7/17 (41)	0.29 (0.10–00.87)	***0.028***
GT/TT	Not BCG treated	13/18 (72)	1.0	
	BCG treated	12/17 (71)	0.54 (0.24–1.20)	0.130

Data are presented as Hazard ratios (HR), adjusted for age, sex and tumor stage and grade, in a stepwise selection model, for each of the *NOS*-polymorphisms.
Number of patients is abbreviated as N.o. Pts.
p-value less than 0.05, are shown in bold and italic.

[a] Since no BCG-treated TT-patients died it was not possible to calculate any hazard ratios.

Table 3
Cancer specific death, disease progression and recurrence in patients with high-risk NMIBC.

		N.o. Pts/total N.o. (%)	Adjusted HR (95% CI)	*p*-Value
CSD				
Basal level NO	Not BCG treated	13/33 (39)	1.0	
	BCG treated	3/36 (8)	0.17 (0.05–0.59)	***0.006***
High/low NO	Not BCG treated	1/4 (25)	1.0	
	BCG treated	5/15 (33)	3.04 (0.35–26.4)	0.314
Progression				
Basal level NO	Not BCG treated	18/33 (55)	1.0	
	BCG treated	6/36 (17)	0.21 (0.08–0.55)	***0.002***
High/low NO	Not BCG treated	2/4 (50)	1.0	
	BCG treated	5/15 (33)	1.34 (0.26–7.01)	0.731
Recurrence				
Basal level NO	Not BCG treated	23/33 (70)	1.0	
	BCG treated	12/36 (33)	0.28 (0.13–00.58)	***0.001***
High/low NO	Not BCG treated	4/4 (100)	1.0	
	BCG treated	10/15 (67)	0.10 (0.02–0.54)	***0.008***

Data are presented as Hazard ratios (HR), adjusted for age, sex and tumor stage and grade, in a stepwise selection model. Basal level NO: patients with at least one of the alleles *NOS2*(CCTTT)non-L-carrier, *NOS3*-786T > C(TT) or Glu298Asp(GG) were considered to yield basal level levels of NO (i.e. neither increased, nor decreased gene activity). High/low NO: patients with the *NOS2*(CCTTT)L-carrier, *NOS3*-786T > C(CT/CC) and Glu298Asp(GT/TT) genotypes were considered to have altered gene activity in both the *NOS2* and the *NOS3* genes.
Number of patients is abbreviated as N.o. Pts.

For the *NOS3*-rs2070744 polymorphism, the C-allele has been shown to reduce the *NOS3* promoter activity and it has been associated with decreased levels of serum nitrite/nitrate and endothelial NO-production in humans [22]. Furthermore, overexpressing *NOS3* in the breast cancer cell line MCF-7 resulted in elevated basal NO levels, which stimulated apoptosis and diminished cancer cell invasion [23]. This indicates that a decreased *NOS3* activity could increase the risk for developing cancer, which is consistent with our earlier data where an association was found between bladder cancer development and homozygous carriers of the C-allele [12]. Also in the *NOS3* rs1799983 polymorphism it has been shown that carriers of the less common T-allele have a lower level of nitrate/nitrite in urine and a lower NOS activity than carriers of the GG genotype [24].

Our study implicates that patients with the *NOS3* polymorphism genotypes rs2070744 (TT) and rs1799983 (GG) responded better to BCG treatment than those with the *NOS3* genotypes that theoretically decrease *NOS3* activity.

Combined analyses of all three *NOS*-polymorphisms showed that patients with genotypes that neither increased *NOS2* gene activity, nor decreased *NOS3* gene activity (i.e. patients with basal level gene activity in both the *NOS2* and *NOS3* gene) had the best response to BCG-treatment. For the subgroup of BCG-treated patients (15/51, approximately 30%) who were not homozygous for at least one of the alleles associated with basal level gene activity (*NOS2* (CCTTT)non-L-carrier, *NOS3*-rs2070744 (TT) or rs1799983

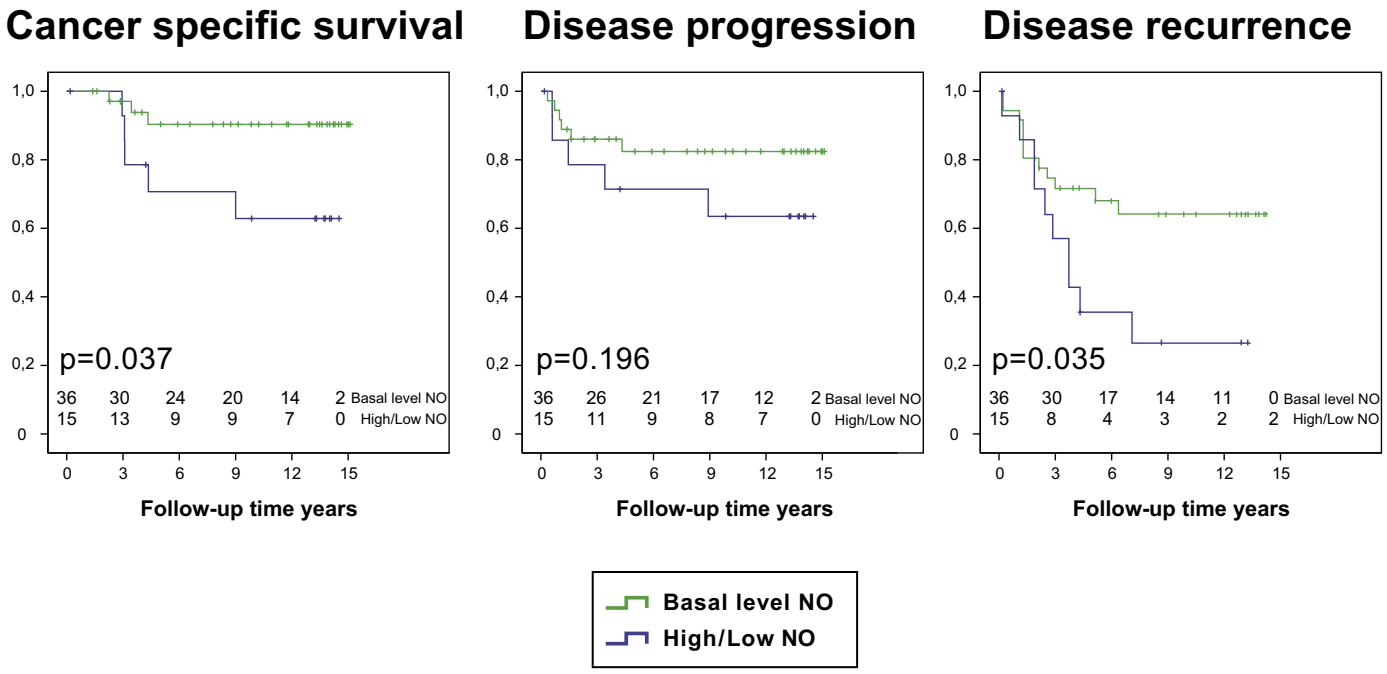

Fig. 1. Difference in survival after BCG-treatment. Comparison within the group of the BCG-treated patients ($n=51$), between those with at least one of the *NOS2*(CCTTT) non-L-carrier, *NOS3*-rs2070744(TT) or rs1799983(GG) considered to yield basal levels of NO (i.e. neither increased *NOS2* gene activity, nor decreased *NOS3* gene activity) ($n=36/51$; 70.6%; Basal level NO), and those with the *NOS2*(CCTTT)L-carrier, *NOS3*- rs2070744(CT/CC) and rs1799983(GT/TT) genotypes, who in theory have increased *NOS2* gene activity and decreased activity in the *NOS3* gene ($n=15/51$; 29.4%; High/low NO). Patients still at risk are shown for each time point in the diagrams. (A) BCG-treated patients with basal level of *NOS*-gene activity showed a significantly better cancer specific survival (Log-rank test: $p=0.037$), than BCG-treated patients with increased *NOS2* and decreased *NOS3*-gene activity. (B) The difference in risk of disease progression between the two groups of BCG-treated patients with and without basal level of *NOS*-gene activity was non-significant (Log-rank test: $p=0.196$). (C) BCG-treated patients with basal level of *NOS*-gene activity had a significantly lower risk of recurrence (Log-rank test: $p=0.035$), than BCG-treated patients with increased *NOS2* and decreased *NOS3*-gene activity.

(GG)), BCG-therapy could not be shown to be beneficial.

Many studies have previously discussed possible genetic biomarkers for BCG-failures [25–27] to help distinguish patients who would require a more aggressive initial treatment than BCG. Recently, Kang et al. investigated *GSTM1* and *GSTT1* polymorphisms and found an association with early BCG-failure [27]. However, so far no exclusively convincing markers have been identified. Combining the different findings of genetic polymorphisms suggested to influence the outcome after BCG-treatment would be interesting. Our findings, together with those of others, may help determine whether patients would benefit from BCG-treatment, or if more aggressive treatment may be required.

This is a population-based study with a long follow-up, including all patients with high-risk NMIBC where normal tissue was available for genotyping, yet the study population is of limited size. Since the study is of prospective design, there has been no randomization to treatment, and regional traditions and physicians' choices decided whether the patient received BCG or not. However, the numbers of patients in the BCG-treated versus the non-BCG treated group were approximately the same. We did not measure the concentration and activity of the NOS-enzymes directly in the patients, but instead relied on the association between these polymorphisms and altered enzyme activity previously shown in many studies [20–23,28]. A limitation of this study is that interethnic differences in polymorphic frequencies may limit the applicability of our findings to populations of Caucasian origin [29].

5. Conclusion

Radical surgery in BCG-non-responders often results in a less favorable prognosis than in those patients undergoing immediate radical cystectomy. Using polymorphisms as prognostic markers may be a possible way to select those high-risk NMIBC-patients who will be at risk of BCG-failure, and thus allow earlier initiation of alternative treatments in this set of patients. Using the *NOS2* (CCTTT)non-L–carrier (homozygous), *NOS3*-rs2070744 (TT) and rs1799983 (GG) genotypes as inclusion criteria for selecting patients for BCG-treatment, only 71% of the BCG-treated patients in the present cohort would have been selected. Within the subgroup of BCG non-responders, immediate radical cystectomy instead of BCG as primary treatment could probably have increased the survival rate, had we known how to identify these patients at an earlier stage. Although our results need to be confirmed in larger studies that validate the effects of *NOS*-polymorphisms on the outcome after BCG-treatment, our study indicates that the *NOS2* (CCTTT)n promoter polymorphism and the two *NOS3* polymorphisms, rs2070744 and rs1799983, may predict the outcome of BCG-treatment in high-risk NMIBC-patients, and as such may serve as possible molecular markers to identify patients suitable for BCG-treatment.

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