European Journal of Cancer 59 (2016) 134–141

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Variation in the *HFE* gene is associated with the development of bleomycin-induced pulmonary toxicity in testicular cancer patients

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Received 30 September 2015; received in revised form 5 January 2016; accepted 13 February 2016
Available online 28 March 2016

KEYWORDS
Testicular neoplasms;
Survivors;
Antineoplastic agents;
HFE;
Bleomycin;
Cisplatin;
Toxicity;
Variation

Abstract ***Background:*** Bleomycin and cisplatin are of key importance in testicular cancer treatment. Known potential serious adverse effects are bleomycin-induced pulmonary toxicity (BIP) and cisplatin-induced renal toxicity. Iron handling may play a role in development of this toxicity. Carriage of allelic variants of the *HFE* gene induces altered iron metabolism and may contribute to toxicity. We investigated the association between two common allelic variants of the *HFE* gene, H63D and C282Y, with development of pulmonary and renal toxicity during and after treatment with bleomycin- and cisplatin-containing chemotherapy.
Methods: In 369 testicular cancer patients treated with bleomycin and cisplatin at the University Medical Center Groningen between 1978 and 2006, H63D and/or C282Y genotypes were determined with an allelic discrimination assay. Data were collected on development of BIP, pulmonary function parameters, renal function, and survival.
Results: BIP developed more frequently in patients who were heterozygote (16 in 75, 21%) and homozygote (2 in 4, 50%) for the H63D variant, compared with those who had the *HFE* wild-type gene (31 in 278, 11%) ($p = 0.012$). Overall survival, testicular cancer-related survival, and change in renal function were not associated with the H63D variant.
Conclusion: We observed an association between presence of one or both H63D alleles and development of BIP in testicular cancer patients treated with bleomycin combination chemotherapy. In patients heterozygote and homozygote for the H63D variant, BIP occurred more

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http://dx.doi.org/10.1016/j.ejca.2016.02.013

frequently compared with wild-type patients. When validated and confirmed, *HFE* H63D genotyping may be used to identify patients with increased risk for pulmonary bleomycin toxicity.

1. Introduction

Testicular germ-cell cancer is the most frequently diagnosed cancer type among young men between the ages of 18 and 35 [1]. Introduction of bleomycin- and cisplatin-containing chemotherapy dramatically improved survival of patients with metastatic disease [2]. Unfortunately, a significant number of testicular cancer patients develop toxicity induced by bleomycin and cisplatin, both during and after completion of treatment [3]. Important adverse events are bleomycin-induced pulmonary toxicity (BIP) and cisplatin-induced renal toxicity. Signs of BIP occur in roughly 10% of the patients receiving bleomycin and may be fatal in 1–3% of these patients [4,5]. Renal toxicity is both an acute and late adverse effect of cisplatin-containing chemotherapy for testicular cancer [6] and is largely prevented by adequate hydration.

The toxic effect of bleomycin on tumour tissue and on healthy tissue can be attributed to its ability to initiate single- and double-strand DNA breaks in the presence of iron (Fe^{2+}) and oxygen (O_2) [7,8]. Bleomycin can bind to DNA by forming a complex with Fe^{2+} in presence of O_2, inducing generation of extremely reactive hydroxyl radicals, which induce DNA damage [8,9]. These hydroxyl radicals also lead to apoptosis of healthy epithelial and endothelial cells, although this is restricted due to protective activity of the bleomycin-degrading enzyme bleomycin hydrolase (BLMH) [10]. However, in pulmonary tissue, BLMH expression is low. Subsequently, lungs are prone to develop BIP [7].

Accumulation of iron occurs in patients with hereditary haemochromatosis, a genetic disorder resulting in altered iron metabolism caused by variants of the *HFE* gene [11]. In idiopathic pulmonary fibrosis, carriage of variants in the *HFE* gene, H63D and C282Y, leads to excessive accumulation of extracellular iron in lungs [12]. Also, iron-dependent generation of reactive oxygen species (ROS) increased in carriers of *HFE* gene variants. It is suggested that this altered iron homoeostasis leads to microscopic oxidative stress-induced pulmonary injury [12].

Iron may also be involved in development of cisplatin-induced renal toxicity. Exposure of cisplatin to renal tubular epithelial cells increases iron levels and the amount of ROS in *in vitro* and *in vivo* models [13]. Addition of iron chelators could prevent cisplatin-induced cytotoxicity and provides protection against cisplatin-induced renal failure [13].

The interaction of iron with bleomycin and cisplatin handling suggests that variation in the *HFE* gene might also contribute to development of pulmonary and renal toxicity. There is an unmet need to identify patients susceptible for serious BIP and cisplatin-induced renal toxicity. Therefore, the aim of this study was to investigate the association between two common allelic variants of the *HFE* gene, H63D and C282Y, with development of pulmonary and renal toxicity in testicular cancer patients treated with bleomycin- and cisplatin-containing chemotherapy.

2. Patients and methods

2.1. Study population

Patients with metastatic testicular cancer treated with bleomycin- and cisplatin-containing chemotherapy at the University Medical Center Groningen (UMCG) between 1978 and 2006, aged 16 years or older, from whom genomic DNA was available for genotyping were eligible. After informed consent, genomic DNA was isolated from peripheral blood samples collected into EDTA tubes at the general practitioner or at the outpatient clinic. For deceased patients, genomic DNA was isolated from stored serum samples when available [14,15]. The study protocol was approved by the local medical ethics committee.

2.2. Baseline characteristics

The following baseline characteristics were obtained from patient's medical records: age at start of chemotherapy, Royal Marsden stage, International Germ Cell Cancer Collaborative Group (IGCCCG) classification, presence of pulmonary metastases, chemotherapeutic regimen, cumulative bleomycin and cisplatin dosages, and creatinine clearance (CRCL).

2.3. Endpoints

2.3.1. Bleomycin-induced pulmonary toxicity

Occurrence of BIP was examined through patients' medical records. Severity of BIP was classified as follows: (a) death due to BIP, (b) clinical and/or radiologic signs of BIP, resulting in hospitalisation or early discontinuation of bleomycin administration, (c) clinical and/or radiologic signs of BIP after completion of treatment, and (d) no signs of BIP [15]. Classification of