Genetic alterations in periprosthetic soft-tissue masses from patients with metal-on-metal hip replacement

Virinder Kaur Sarhadi a, Jyrki Parkkinen b, c, Aleksi Reito b, Jyrki Nieminen b, Noora Porkka a, Tiina Wirtanen a, d, Minna Laitinen b, Antti Eskelinen b, Sakari Knuutila a, * a University of Helsinki, Faculty of Medicine, Department of Pathology, Helsinki, Finland b Cox Hospital for Joint Replacement, Tampere, Finland c Department of Pathology, FIMLAB Laboratories, Tampere, Finland d HUSLAB, Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland

A R T I C L E   I N F O

Article history:
Received 23 April 2015
Received in revised form 23 July 2015
Accepted 27 August 2015
Available online 29 August 2015

Keywords:
Periprosthetic tissue
Hip replacement
Pseudotumor
Mutations
aCGH
Metal-on-metal implant

A B S T R A C T

Adverse soft tissue reactions in patients with metal-on-metal (MoM) hip replacement are associated with cobalt (Co) and chromium (Cr) particles released from the implant. Exposing the patients to long periods of increased metal ions concentrations resulting from the wear of these implants poses an increased risk of genotoxicity/mutagenicity. A variable proportion of patients develop periprosthetic soft-tissue masses or pseudotumors at the site of the implant. There is a concern that exposure to increased metal ions could increase the risk of cancer. In order to investigate whether the periprosthetic soft-tissue mass harbours any cancer-related genetic alterations, we studied DNA isolated from periprosthetic tissues of 20 patients with MoM hip replacement, for copy number alterations and mutations in hotspot regions of 50 cancer genes using aCGH and amplicon-based next generation sequencing.

Our results showed copy number gains at 12q14.3 and 21q21.1 in tumour from patient diagnosed with liposarcoma. Copy number alterations in periprosthetic tissues were seen in three other patients, one had a region of gain at 9q24.1 affecting JAK2 and INSL6, and two patients had region of gain at 6p21.1, affecting RUNX2. Mutation analysis showed V1578del mutation in NOTCH1 in two patients. The copy number alterations and mutations seen in periprosthetic soft-tissue masses are earlier reported in either haematological malignancies or in osteoblast related bone dysplasia. The presence of genetic anomalies was associated with longer in-situ time of the implant. Our findings warrant the need of similar studies in larger patient cohorts to evaluate the risk of development of neoplastic alterations in periprosthetic tissues of patients with MoM hip replacement.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Higher than anticipated revision rates in metal-on-metal (MoM) hip replacements have prompted medical device alerts by the authorities [1–4]. Adverse reactions to metal debris (ARMD) are thought to be the underlying reason for the increased failure rates of metal-on-metal hips. ARMD is an umbrella term for periprosthetic soft tissue reactions induced by metal particles released from the bearing surfaces and from the head–neck junction in MoM hips [5]. One clinical manifestation of such adverse soft tissue reaction is a pseudotumor, which means a periprosthetic soft-tissue mass originating from the hip joint [6]. Other manifestations include synovitis, soft-tissue necrosis, metallosis and osteolysis in the periprosthetic tissues. Incidence of ARMD ranges from 0 to 31% in patients with MoM hip replacements [7,8]. Increasing incidence of ARMD has led to abandonment of stemmed MoM hip replacements worldwide. MoM hip resurfacing, however, which is mainly being used is young male patients, is under heavy scrutiny but has not yet been abandoned globally.

A long term exposure to metal products can have genotoxic or carcinogenic effects. Increased numbers of chromosomal aberrations have been reported in peripheral blood of patients after MoM hip replacement [9]. Moreover in vitro studies have shown genotoxic effect of metal particles on human fibroblasts, including increased number of chromosomal numerical changes [10].

The risk of cancer development in patients with hip replacement is doubtful. Although large population based studies [11,12] have
not found any overall increased risk of cancer associated with MoM implants, an increased risk of soft tissue sarcoma and basalioma in patients with MoM implants is reported in a Finnish registry-based study [12].

There are no studies related to the genetic changes examined from periprosthetic tissues of patients with MoM hip replacement. In order to investigate whether the periprosthetic tissues show any genetic alterations of neoplastic significance, we studied DNA extracted from these tissues of patients undergoing hip revision surgery because of ARMD. These DNAs were analysed for copy number alterations (CNA) by array CGH and mutation analysis of hotspot regions of 50 cancer genes by amplicon-based next generation sequencing.

2. Materials and methods

A total of 20 patients (9 males and 11 females) with a MoM hip replacement, who underwent hip revision surgery because of ARMD at our institution were included in the study. Three of the patients had incidental lipomatous lesions in magnetic resonance imaging (MRI), which were in connection with the periprosthetic reactive tissue. Marginal resection of these soft tissue tumours was performed simultaneously with revision surgery of the MoM hip replacement. Detail description about patients is summarized in Table 1. ALVAL (aseptic lymphocyte vasculitis-associated lesions) was used to describe the severity of histological reaction in the synovia. The ALVAL-score with 10-point scale was used semi-quantitatively to rank synovial lining integrity, inflammatory cell infiltrates and tissue organization [13]. The study was approved by the “Ethics Committee of Pirkanmaa Hospital District”, permission number: R11006 and R11196.

2.1. Blood metal ion analysis

19 patients underwent whole blood analysis of cobalt (Co) and chromium (Cr) following sampling from the antecubital vein using a twenty-one-gauge needle connected to a Vacutainer™ system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and trace element blood tubes containing sodium ethylenediaminetetraacetic acid. In the Finnish Institute for Occupational Health, standard operating procedures were established for cobalt and chromium measurement using dynamic reaction cell inductively coupled plasma (quadrupole) mass spectrometry (ICPMS) (Agilent 7500 cx, Agilent Technologies, Santa Clara, CA, USA).

2.2. Cross-sectional imaging

Magnetic resonance imaging (MRI) was performed with two 1.5T scanners (Siemens Magnetom Avanto, Siemens Healthcare, Erlangen, Germany and GE Sigma HD, General Electric, Healthcare, Wisconsin, USA). Metal artefact reduction sequence was used in MRI: coronal and axial T1 weighted fast spin echo and coronal, axial and sagittal short tau inversion recovery. MRI findings were categorized using an Imperial classification. Ultrasound (US) examinations were performed with Logiq e9 (GE Healthcare, Wisconsin, USA) and graded by the same musculoskeletal radiologist.

2.3. Definition of ARMD

Diagnosis of adverse reactions to metal debris was based on perioperative findings [8]. Failure was classified as being secondary to adverse reactions to metal debris if the following criteria were met: (1) there was presence of metallosis or macroscopic synovitis in the joint; and/or (2) a pseudotumour was found during revision; and/or (3) a perivascular lymphocyte infiltration graded many to excessive in a Willert scale was seen in the histopathology sample [14]; and (4) perioperatively there was no evidence of component loosening or periprosthetic fracture. Furthermore, infection was ruled out by multiple (at least five) bacterial cultures obtained during revision surgery.

DNA was isolated from formalin-fixed paraffin-embedded (FFPE) sections of periprosthetic soft-tissue masses collected at our institution, using QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany), following manufacturer’s instructions. DNA from blood was isolated by enzymatic method [15]. DNA concentration was measured using Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and with NanoDrop-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

2.4. Array CGH

DNA samples and gender-matched reference DNA were digested, labelled, hybridized and washed according to Agilent’s Oligonucleotide Array-Based CGH for Genomic DNA analysis protocol version 6.1 (Agilent Technologies, Santa Clara, CA, USA). Briefly, twoug of DNA were first digested with Alu1 and RsA1 restriction enzymes. Digested patient DNA was labelled by priming with Cy-5dUTP and reference DNA with Cy3-dUTP by using Agilent Complete SureTag Complete DNA Labelling Kit (Agilent Technologies).

The labelled samples were hybridized on Agilent’s Human Genome CGH Microarray 1 x 44K oligonucleotide platform (Agilent Technologies). After hybridization, the microarrays were scanned on Agilent’s scanner. Feature extraction software v11.5.1.1. (Agilent Technologies) was used to extract the image data and the analysis was completed with Agilent CytoGenomics v2.0.6.0 Software (Agilent Technologies) using aberration detection method ADM-2 algorithm with sensitivity threshold of 6.0. To check the somatic nature of genetic changes, aCGH was also performed on DNA isolated from normal synovial tissue or blood of two patients with genetic alterations.

2.5. NGS

Ten ng of DNA were used to prepare the barcoded libraries with the Ion AmpliSeq™ Library kit 2.0 (Life Technologies). The HotSpot Cancer panel V2 (Life Technologies) was used to analyse around 2800 COSMIC mutations in 50 genes (ABL1, AKT1, ALK, APC, ATM, BRAF, EGFR, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, IDH2, JAK3, KDR, KIT, KRAS, MET, Pten, PTPN11, RB1, RET, SMAD4, SMARCB1, CDH1, GNA11, MLH1, SMO, CDKN2A, GNAS, MPL, SRC, CSF1R, GNAQ, NOTCH1, STK11, CTNNB1, HNF1A, NPM1, TP53, HRAS, NRAS, VHL, ERBB2, IDH1, PDGFRα, JAK2, PIK3CA). The barcoded libraries from 8 patients were pooled together. Template preparation and enrichment was performed with the Ion OneTouch™ 2 System (Life Technologies). Finally, sequencing was carried out using Ion 316™ chips on the Ion Personal Genome Machine System (PGM™, Life Technologies) and with the Ion PGM™ Sequencing 200 kit v2.

Alignment to the hg19 human reference genome and variant calling was performed by the Torrent Suite Software v4.0.2 (Life Technologies). Alignments were visually checked with the Integrative Genomics Viewer: IGV v2.3, Broad Institute [16].

Statistical analyses were carried out using IBM SPSS version 22 software. Correlation of blood metal ion levels with age, ALVAL score, in-situ time of implant was analysed using Spearman’s correlation coefficient. Association of genetic alterations with age, gender, ALVAL score, in-situ time of implant, and blood metal concentration in patients was studied using Mann–Whitney U test.

3. Results

Median age of the patients included in the study was 59 yrs (45–70 yrs) with an average of 4.7 years between
primary hip replacement and revision surgery. Spearman correlation coefficient/Mann–Whitney analysis did not show any significant correlation between the levels of metal ions (chromium and cobalt) in whole blood and age, sex, or in-situ time of the MoM hip replacement. Histological evaluation of the periprosthetic soft-tissue from 20 patients revealed liposarcoma in one patient and lipoma in two patients.

Nine out of 20 patients had a mixed-type pseudotumor (thick-walled atypical fluid) in pre-revision MRI. One patient had a pseudotumor with same characteristics in pre-revision US. One patient had a joint effusion without a pseudotumor in pre-revision US. Four patients did not have any abnormal findings in pre-revision MRI. Three patients were not imaged before revision surgery. In all patients, perioperative findings encountered during hip revision surgery were consistent with an adverse local tissue reaction. Further, histopathological examination of periprosthetic tissues confirmed the clinical diagnosis in all patients. ALVAL-scores are shown in Table 1.

### 3.1. Array CGH

Genome-wide copy number analyses performed on DNA from periprosthetic soft-tissue revealed CNAs in four patients, one with histological classification of liposarcoma and in three others with no detectable neoplastic changes in histology (Table 1). Both the patients with lipoma did not show any CNA.

#### 3.1.1. Gain at 12q14.3 and 21q21.1

aCGH analysis from periprosthetic tissue of patient with liposarcoma, showed two regions with complex pattern of gains at 12q14.3 and 21q21.1 affecting many genes (Fig. 1). The gain at 12q14.3 (Chr12:67,794,618–71,466,258) encompassing a ~3.6 Mb region affects 26 genes including MDM1 and MDM2. Additional small regions of gain at 12q14.3 were: 68,656 bp including RPSAP52, HMG2; 88,150 bp including TMBIM4, IRAK3; and at 12q21 were: 238,079 bp including PTPRR; 271,242 bp including LIPI, RBM11, ABCC13, HSPA13, G100549.

The second region of CNA on chromosome 21 also showed a complex pattern. A number of genes are located in this region. A 14.7 Mb gain at 21q21.1 (Chr21:16,390,025–31,129,773) encompassed 40 genes, including many MIRNAs; five smaller regions of gain at 21q22 and one region of copy number loss. The copy number alterations were confirmed to be somatic since no CNA was seen in normal synovial tissue from the same patient.

#### 3.1.2. Gain at 9p24.1

The second patient with copy number alterations (Patient no. 1) had a small 0.23 Mb region of gain at 9p24.1 (chr9:970,093–5,203,776) encompassing JAK2 and INSL6 genes seen in periprosthetic tissue (Fig. 1B). The somatic nature of this alteration could not however be inferred as no normal tissue was available from this patient.

#### 3.1.3. Gain at 6p21.1

A 0.13Mb gain encompassing RUNX2 at 6p21.1 (chr6:45,383,847–45,515,349) was observed in two patients. The change was confirmed to be somatic in one patient (Fig. 1C), while aCGH could not be performed on any normal tissue in the other patient.

### 3.2. NGS

Five out of a total of 20 patients had a missense or deletion mutation at a hotspot position in one of the genes of the cancer gene panel (Table 1). All of the 3 mutations in NOTCH1, KIT and MET have previously been reported in COSMIC database [17]. A V1578del mutation in NOTCH1 was seen in periprosthetic tissue from two patients and a homozygous MET T1010I was seen in one patient. Both these mutations are earlier reported to be somatic. Two patients had KIT M541L mutation, which is reported both as somatic and germline. To check the somatic status of NOTCH1 and MET mutations, sequencing was repeated for these patients, and also performed on DNA isolated from blood of these patients. MET T1010I showed homozygous pattern in both tumour and blood sample, indicating its clear germline origin. Patients with NOTCH1 V1578del in periprosthetic tissue also showed this mutation in low frequency (in around 1% of reads) in DNA from blood of these patients.

Patients with genetic alterations (CNA or mutations) had significantly longer in-situ duration of implant (i.e. time since the primary hip surgery and revision surgery) as analysed by Mann-Whitney test (mean rank = 8.79 and 14.50 for patients without genetic alter-
Fig. 1. Copy number alterations as seen by array CGH in DNA from periprosthetic tissue of patients with MoM hip replacement. A. aCGH shows a gain at 12q1.1 and a complex pattern of gain at 21q21.1 in tumour DNA and no CNA in normal tissue from patient 7 with liposarcoma. B. A gain at 9p24.1 affecting JAK2 seen in periprosthetic tissue of patient 1. C. A CNA at 6p21 seen in periprosthetic tissue of patient 12 leading to gain of RUNX2.

4. Discussion

This is the first study to report genetic alterations in periprosthetic tissues from patients with MoM hip replacements. In an attempt to investigate whether the periprosthetic tissues harbour any neoplastic alterations at the DNA level, we studied copy number alterations and mutation analysis of cancer genes in periprosthetic tissues of patients with MoM hip replacements that had failed due to ARMD. Our results showed somatic genetic alterations in six out of the 20 patients investigated. A patient with liposarcoma showed two regions of CNA with genetic instability at 12q14.3 and 21q21.1 loci (Fig. 1A). No mutations in the cancer genes analysed were seen in this patient. Gain at 12q is the most frequent CNA seen in synovial sarcomas as reported earlier by our group [18] and in liposarcomas [19]. The region of complex CNA at 21q21 harbours nearly 40 genes including many important miRNA e.g. MiR99A, MiR177C, MiR125B2, MiR155HG, MiR155. MiR-155 acts as oncogene and is the most overexpressed miRNA in liposarcoma [20].

A region of gain at 9p24.1 seen in periprosthetic tissue of a patient with normal histology encompasses JAK2 and INSL6 (Fig. 1B). A similar overlapping 9p24.1 amplification including PD-L1, PD-L2 and JAK2 is a recurrent alteration in 40% of primary nodular sclerosing Hodgkin lymphomas and in 60% of primary mediastinal large B-cell lymphomas [21]. JAK2 is a protein Kinase involved in cytokine receptor signalling pathways. Increased JAK2 signalling is reported to play a role in growth and maintenance of acute myeloid leukaemia stem cells [22] and mutations in JAK2 are frequently seen in myeloproliferative neoplasms.

The third region of gain at 6p21.1 seen in periprosthetic tissue of two patients, affected RUNX2 (Fig. 1C). A gain of function, 105-kb duplication comprising exons 1–5 of the RUNX2 gene causes metaphyseal dysplasia with maxillary hypoplasia and brachydactyly [23]. The gain seen in our study covers the whole of shorter transcript variant (Type II RUNX2 mRNA) and exon 3 to last exon (8 or 9) of the longer Type I transcript. RUNX2 is a transcription factor that plays a very essential regulatory role in osteoblast differentiation and expression of osteoblast-specific genes. Mutations in this gene cause cleidocranial dysplasia, a rare autosomal dominant skeletal dysplasia [24].

The most frequent somatic mutation in NOTCH1, seen in 2 patients was a 3 bp deletion leading to in-frame deletion of single amino acid valine at codon 1578 (COSM13047). This mutation is frequently seen as somatic mutation in leukaemia patients, especially T-cell leukemia [25,26]. Our results showed that in addition to the presence of this mutation in the periprosthetic tissue, it was also seen in very low frequency in blood DNA. Since inflammatory cells, namely CD3 positive T-lymphocytes and CD68 positive macrophages, are usually seen in the periprosthetic tissues, it is
quite possible that a small clone of blood cells harbour this mutation, and these cells are more abundant in the periprosthetic tissue. More so because this mutation is earlier reported mainly in neoplastic cells of lymphoid origin [25,26] and there are earlier reports of chromosomal abnormalities seen in blood cells from patients with MoM hip replacement [5].

MET T1010I mutation, reported as somatic mutation in COSMIC and dbSNP database is seen largely in lung cancer patients [27] and thyroid carcinomas (according to COSMIC database) and recently also in Ewing’s sarcoma [28]. MET, is a proto-oncogene and receptor tyrosine kinase that is implicated in tumour growth and metastasis The T1010I mutation is located in the juxtamembrane domain, which is essential for catalytic function of receptor tyrosine kinases. This mutation is homoygous with germline origin in our patient, so its role in periprosthetic tissue is not clear. Similarly, the role of KIT M541L mutation seen in 2 of our patients in pseudotumor development is also doubtful since it is also seen in normal population, although its somatic origin is reported in acute myeloid patients and also in stomach adenocarcinoma (according to COSMIC database).

In the periprosthetic tissues of patients with failed MoM hip replacements, we found such genetic changes in the DNA that have earlier been reported mainly in cells of haematopoietic/lymphatic origin (JAK2, NOTCH1) or osteoblasts (RUNX2). The effect of metal ions on both these types of cells is well documented in previous studies as reviewed by Mabileau and colleagues [29]. A T-lymphocyte-mediated hypersensitivity reaction of type IV (delayed-type hypersensitivity) resulting from the metal-wear particulate debris from the MoM bearing [30] and in vitro toxic effects of cobalt and chromium on osteoblasts including dysregulation of expression of antioxidant enzymes is reported [31]. In vivo studies have found increased incidence of sarcomas and lymphomas with bone involvement associated with metal implants [32] and elevated risk of myelodysplastic syndrome probably associated with exposure to metal is reported in a long follow-up study of knee replacement patients [33]. Two patients with lipoma in our study showed no CNA, while one of them had NOTCH1 mutation.

We did not find any significant association of genetic alterations with levels of metal ions in whole blood, though the cobalt levels in all patients were very high compared to other studies (mainly due to our patient selection bias); median value of 18.0 for Cobalt and 11.0 for Cr (excluding patients with bilateral implants), compared to 8.3 μg/L and 5.9 μg/L in patients with pseudotumors, and 1.0 μg/L and 1.3 μg/L without pseudotumors, respectively, reported in other studies [34]. However increased in-situ time of implant was found to be significantly associated with genetic alterations. A recent study has shown a significant increased incidence of pseudotumors after prolonged follow-up and with no clear association of cobalt or chromium levels with pseudotumor occurrence [35].

The association of genetic damage with longer in-situ time of implant and absence of any significant association with metal ion concentration could indicate prolonged inflammatory response to particulate metals as the probable cause of genetic damage rather than the genotoxic effect of solubilized metal ions as reported earlier [9]. Persistent inflammation is reported to increase accumulation of genetic alterations mediated by transcription-associated mutagenesis via cytidine deamination activity of activation-induced cytidine deaminase (AID) in liver tissue [36]. Studies comparing MoM and non-MoM implants are however needed to see if implant time and chronic inflammation account for the presence of genetic alterations.

As MoM hip replacements have been implanted in around 1.5 million people worldwide and MoM hip resurfacings are still used in young patients with hip osteoarthritis, there is obvious need for further research in larger cohorts of patients to evaluate whether these devices are associated with an elevated risk of neoplastic alteration.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Acknowledgement

The study was supported by grants from “The Competitive Research funds of Pirkanmaa Hospital District, Tampere, Finland”.

References


