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Benzene poisoning, clinical and blood abnormalities in two Brazilian female gas station attendants: a case report --Manuscript Draft--

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Article Type:	Case Report
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**Benzene poisoning, clinical and blood abnormalities in
two Brazilian female gas station attendants: a case report**

Fábio Santiago ^{1,2,3}	fabiosantiago.uerj@gmail.com
Simone Lima ¹	simonemattioda@gmail.com
Tayná Pinheiro ¹	tayna.meduerj@gmail.com
Rafaele Tavares Silvestre ^{1,2,3}	rafaelesilvestre@gmail.com
Ubirani Barros Otero ⁴	uotero@inca.gov.br
Marianne Medeiros Tabalipa ⁴	mtabalipa@yahoo.com
Nadezda Kosyakova ⁵	NADEZDA.KOSYAKOVA@med.uni-jena.de
Maria Helena Ornellas ^{1,2}	mariahelenaornellas@gmail.com
Thomas Liehr ⁵	Thomas.Liehr@med.uni-jena.de
Gilda Alves ^{1,2,3}	galvesbrown@gmail.com

1- Laboratório de Marcadores Circulantes, Departamento de Patologia e Laboratórios, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

2- Pós-graduação em Ciências Médicas (PGCM), Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

3- Coordenação de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, Brazil.

4- Unidade Técnica de Exposição Ocupacional, Ambiental e Câncer, Coordenação de Prevenção e Vigilância, Instituto Nacional de Câncer, Rio de Janeiro, Brazil.

5- Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Kollegien-gasse 10, D-07743 Jena, Germany

Corresponding author: Maria Helena Ornellas. Address: Avenida Professor Manuel de Abreu 444, Vila Isabel, Rio de Janeiro, Brasil. Zip code 20551-030. Faculdade de Ciências Médicas, Departamento de Patologia Geral, 4º andar. Phone: (55) 21.2868-8047.

Email: mariahelenaornellas@gmail.com

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34 **Keywords:** Benzene, toluene, xylene, cytogenetic, painting chromosome, natural killer.
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1. Background

Brazilian gas station workers are chronic exposed to BTX (benzene, toluene and xylene), mainly benzene, during the working time [1]. Chronic exposure to benzene may lead to progressive degeneration of bone marrow, aplastic anemia and/or leukemia. Genetic damages caused by benzene include sister chromatid exchanges, DNA cross linking agents, DNA adduct formations, and impairment of DNA repair mechanisms [2].

Since 2010, 115 gas station attendants have been monitored in Rio de Janeiro city, Brazil applying fluorescence *in situ* hybridization (FISH), using whole chromosome painting (wcp) probes for chromosomes 1, 2 and 4. Immunophenotyping by flow cytometric, hemogram, biochemistry based analysis and clinical questionnaire were also conducted. Among the study group, we identified two female gas station attendants with acquired complex chromosomal rearrangements (CCRs) and down regulation of the natural killer (NK) cells.

2. Case presentation

2.1. Case 1

A 25-year-old woman, working 8 hours per day, 6 days a week, for the last 4 years as a gas station attendant had one gestation with miscarriage in the first half of pregnancy. Headache, dizziness irritability, asthenia and normal menstrual cycle were reported. A physical examination showed changes in the thyroid gland, nodules in the right lobe, and a nonspecific pulmonary auscultation. The attendant also reported being a former smoker and not having a family history of cancer. FISH analysis revealed in 1/100 metaphases a CCR involving 8 chromosomal breakpoints described as: 46,XX,der(1)t(1;4),der(4)t(1;4;?),ace(1),ace(1), (Figure 1). Hemogram showed mild neutopenia (1,470 cells/mm³) and biochemistry tests revealed no changes compared to normal values as described in Table I. On the other hand, the immunophenotypic analysis confirmed neutopenia (33.00%), with a lower NK cell count (2.28%), with all NK CD56+/CD16- (Table 2 and Figure 1).

2.2. Case 2

A 40-year-old woman, who was not a smoker or drug addict, working 48 hours a week for the last 9 years as a gas station attendant had a pathological history of one miscarriage in the first

half of pregnancy. Anxiety, dizziness, cramps, asthenia and normal menstrual cycle were reported. No physical examination alteration and no family history of cancer were observed. For case 2, Figure 1 shows one CCR, which was found in 1/100 metaphases, described as der(4)ins(2;4), which was due to a 3 breakpoint event. Complete hemogram and biochemistry tests showed no abnormalities in comparison with normal values, as described in Table I. Like case 1, the immunophenotypic analysis of case 2 resulted in a lower NK cell count (1.51%), with all NK CD56+/CD16- (Table 2 and Figure 1)

3. Conclusions

We characterized cytogenetic, hematological, and immunophenotypic status in two female gas station attendants, who working in gas station with a proved harmful enviromental concentration of BTX. The following abnormalities were found: CCRs, a decrease in NK cells with abnormal CD16 expression, and early pregnancy loss.

It is well known that gas station workers are exposed to potentially harmful chemicals including BTX. However, benzene is considered the main carcinogenic agent (group 1 according to IARC) and studies associate this compound with acquired cytogenetic alterations [3, 4, 5]. Among various forms of benzene-induced genetic alterations, aneuploidy and chromosomal breakage are the most studied [2]. Chromosomal aberrations in peripheral blood lymphocytes of chronically benzene-exposed patients were previously documented [2, 4, 5]. Zhang et al. [2011] reported dose-dependent chromosomal aneuploidies (mono- and trisomies) in the peripheral blood lymphocytes of workers exposed to benzene. In our study, we analyzed only three pairs of chromosomes, which make up 22.8% of the human genome, and chromosomal aberrations (CAs) of high complexities could be detected in 1 out of 100 metaphases per patient (i.e. 1%). The rate of CCRs in normal controls lies between 0 and 0.5%, determined in 1000 metaphases, each [5]. In the present study, only 100 metaphases could be analyzed per case. Thus, the finding of one metaphase with a CCR among 100 cells is at least noteworthy.

Even though CCR detection in peripheral blood is not directly correlated with enhanced cancer risk, it should be kept in mind that such CAs may indicate increased radio- and/or chemosensitivity. As tumors may be induced by environmental factors in combination with a special genetic susceptibility, the two cases reported may be at risk of acquiring malignancies [5].

As is well known, meiosis is a complex process controlled by different checkpoints, but males and females respond differently to meiotic disturbances [6]. During oogenesis, meiosis is generally pursued leading to the formation of aneuploid gametes or with single gene mutations.

Thus, in gametes, acquired genetic changes can be passed on to the next generation. Several epidemiological studies support the idea that genotoxic and nongenotoxic events following benzene exposure may be initiators of childhood leukemia in utero [7]. Another study on AML has shown that disease is usually initiated in utero because the leukemic translocations and other genetic changes are present in blood spots collected at birth [7, 8]. Also interesting is the fact that the majority of the CCR cases are reported in females ascertained through repeated spontaneous abortions or the birth of a malformed child [8].

Besides the detected CCRs pointing towards enhanced chemosensitivity, these two female workers had hematological and immunological abnormalities characterized by mild leukopenia (case 1) and NK abnormalities. There are some studies concerning benzene with hematological and immunological abnormalities in humans [9-13]. The effects of immunotoxicity induced by benzene are depression and alteration of both the immune system mediated by cells and the humoral system [11]. Lan et al. [2004] observed that leucocytes, B and CD4+-T cell counts, were significantly decreased in workers exposed to benzene compared to the controls. In another study, the number of T lymphocytes, lymphocytes T CD4 and T CD8, and NK cells was reduced in the percentages and absolute numbers, and an increase in the monocyte count in workers during the period of exposure was found [12]. Thus, it was suggested that the depressive effect of benzene on the T and NK cells may be a factor of the probable carcinogenic activity of benzene through the immune system.

Natural killer (NK) cells are immune effector cells that recognize both virally infected and malignant target cells. Surprisingly, the results of the immunophenotypic analysis revealed NK CD56 positive (normal fluorescence) and CD16 negative in both cases, suggesting the presence of the rare subtype NK bright in the peripheral blood, which has low cytotoxic action [12]. It is possible that the action of BTX on the immune system had blocked the transition of immature CD56 bright cells into CD56 dim cells and had caused the CAs on chromosome 1 that were described in case 1. Curiously, CD16 gene is located at 1q23 and the CAs could have affected the normal expression of CD16. In agreement with this finding, an 11 year follow up study showed that low NK cytotoxicity of peripheral blood lymphocytes correlates with an increased risk for cancer [13].

The identifications of chromosomal abnormalities and NK downregulation in the blood may be a new indicator for effective follow up of workers exposed to BTX, preventing diseases mainly important for females and their offspring.

4. List of abbreviations

AML: acute myeloid leukemia

BTX: benzene, toluene and xylene

CAs: chromosomal aberrations

CCR: complex chromosomal rearrangements

FISH: fluorescence in situ hybridization

NK: natural killer

5. Declarations

Authors' contributions

GA, UBO and MHO designed the study and applied for Research Ethics Board approval. MMT, TP, RTS and FS recruited the workers. The cytogenetic and immunophenotypic data were analyzed by NK, SL and FS. FS prepared the manuscript draft with important intellectual input from TL and MH. All authors approved the final manuscript and had complete access to the study data.

Acknowledgements

We thank the subjects who volunteered in the study.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All the data supporting our findings is contained within the manuscript.

Consent for publication

All subjects were informed about the nature of the study, the potential benefits, and the risks.

Ethics approval

This study was approved by the local ethics committees (Instituto Nacional de Câncer – INCA (121/09) and Universidade do Estado do Rio de Janeiro – UERJ (758.647), Brazil.

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Tables

Table 1 - Biometrics data (clinical and demographic) of 2 female gas station attendants

Biometrics data	Case 1	Case 2
Smokers	No	No
Ex-smokers	Yes	No
Illicit drug consumption	Yes ^a	No
Alcohol abuse	Yes	No
Family history of cancer	No	No
Blood test		
Erythrocytes (10 ⁶ /μL)	4.0	4.2
Hemoglobin (g/dL)	12.0	12.5
Hematocrit (%)	36.1	38.0
Mean corpuscular volume (fl)	88.7	88.6
Leukocytes(/uL)	4620	7410
Neutrophils(%)	32.1	57.4
Typical lymphocytes (%)	56.7	34.5
Basophils (%)	0.6	0.4
Eosinophils (%)	3.7	1.5
Monocytes (%)	6.9	6.2
Platelets (10 ³ /μL)	230	312
Reticulocytes (%)	1.21	
Gamma-GT (U/L)	31.0	14.0
Oxaloacetic transaminase (U/L)	0,23	17.0
Pyruvic transaminase (U/L)	15.0	11.0
Creatinine (mg/dL)	0.6	0.7
Total bilirubin (mg/dL)	0.23	0.38
Lactate dehydrogenase (mg/dL)	252	320
Rheumatoid fator (IU/mL)	9.6	9.5
C reactive protein (mg/dL)	0.11	0.12

Abraviattions : Gamma GT- Gamma glutamyl transpeptidase. ^amarijuana.

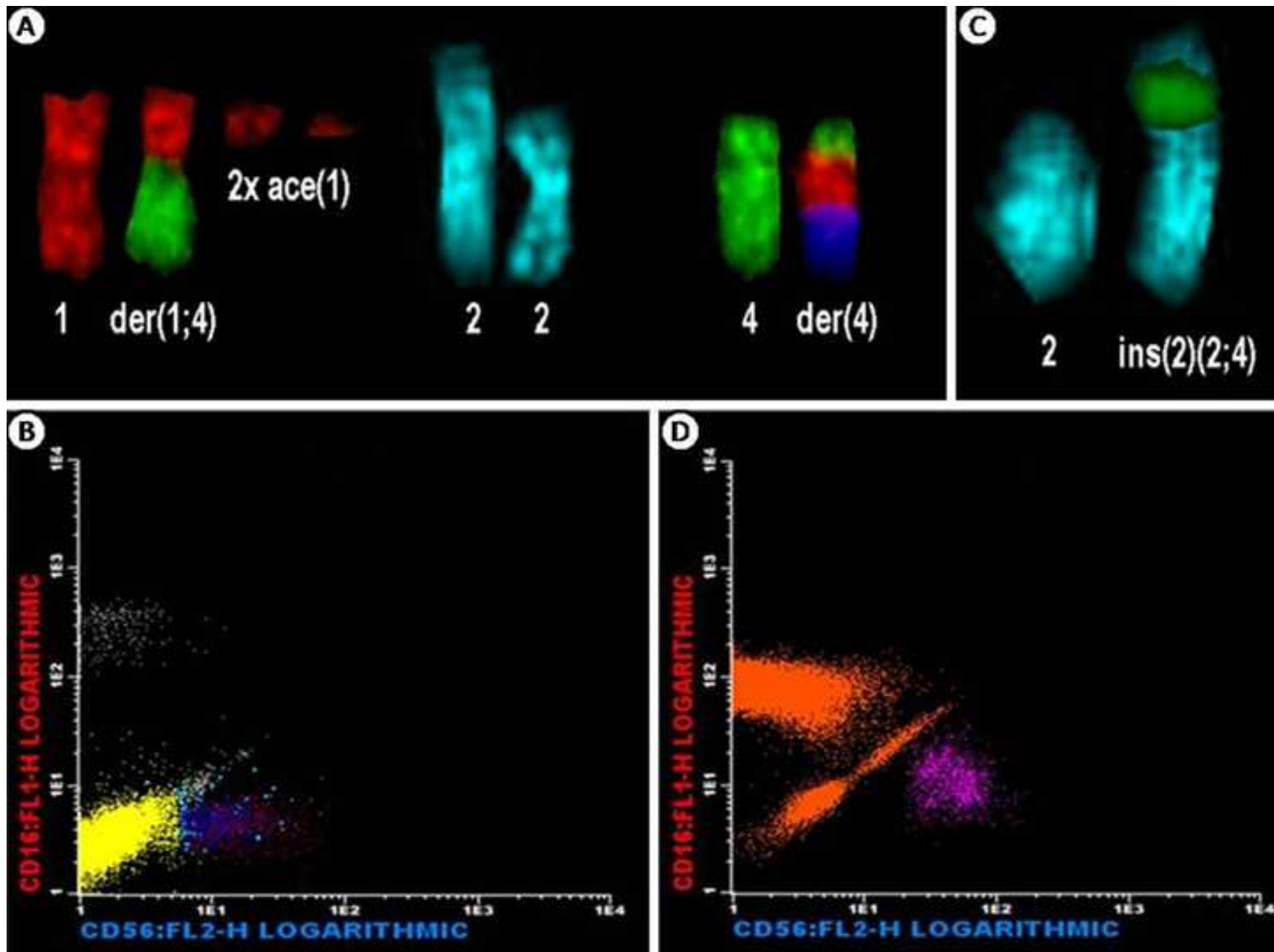
Table 2 - Results of Immunophenotyping by flow cytometry

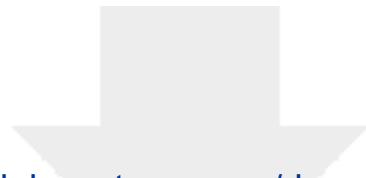
MONOCLONAL	Lympho	Monoc	Granulo	Eosino
Percent of total cells	54.1	6.7	33.00	3.03
CD 4	28.56	-----	-----	-----
CD 8	13.75	-----	-----	-----
CD4 + CD 8	42.31	-----	-----	-----
CD5	44.20	-----	-----	-----
CD7	50.00	-----	-----	-----
CD2	46.60	-----	-----	-----
CD3	41.87	-----	-----	-----
CD16	0.00	0.00	36.5	0.00
CD56	2.28	0.00	0.00	0.00
CD16 + CD 56 (NK)	2.28	0.00	0.00	0.00
CD19	5.77	-----	-----	-----
CD20	6.10	-----	-----	-----
CD10	0.07	-----	28.94	-----
CD27	0.00	0.00	0.00	3.52
CD22	5.88	0.00	0.00	0.00
HLA-DR	7.23	6.42	0.00	4.00
CD11c	3.08	7.06	32.4	3.52
CD25	0.00	0.00	0.00	5.70
Percent of total cells	31.23	5.4	63.6	0.16
CD 4	13.51	-----	-----	-----
CD 8	9.08	-----	-----	-----
CD4+CD8	22.59	-----	-----	-----
CD5	30.85	-----	-----	-----
CD7	25.17	-----	-----	-----
CD2	24.36	-----	-----	-----
CD3	22.59	-----	-----	-----
CD16	0.00	0.00	55.06	0.00
CD56	1.51	0.00	0.00	0.00
CD16 + CD 56 (NK)	1.51	0.00	0.00	0.00
CD19	5.58	-----	-----	-----
CD20	5.59	-----	-----	-----

CD10	0.13	-----	46.09	----
CD27	0.00	0.00	0.00	0.00
CD22	4.88	0.00	0.00	0.00
HLA-DR	4.89	7.12	0.00	0.00
CD 11c	0.20	3.75	49.12	0.00
CD25	0.00	0.00	0.00	0.00

Figure:

Fig.1 CCRs found in case 1 and 2 and flow cytometric analyses of NK cell subsets. Case 1: A, CCR - der(1)t(1;4),der(4)t(1;4;?),ace(1),ace(1). B, flow cytometric analyses of NK cell of case 1. Show all NK cells (CD56 positive) are CD16 negative, thus all are sub-sets of NK^{bright}. Case 2: C, CCR- ins(2;4). Chromosome 2 was folded and thus looks shorter due to preparation. D, flow cytometric analyses of NK cell of case 1. Show all NK cells (CD56 positive) are CD16 negative, thus all are sub-sets of NK^{bright}. A and B, the probes were conjugated with TexasRed to label chromosome 1 (red), Diethylaminocoumarin (DEAC) for chromosome 2 (lightblue) and fluorescein isothiocyanate (FITC) for chromosome 4 (green). Other chromosomes were counter-stained with DAPI (dark blue). C and D, BD FACSCanto II cytometer was used in both cases and the data analyses were performed using the Infinicyt® software.





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Supplementary Material

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