Metallomic Profile in Non-cirrhotic Hepatocellular Carcinoma Supports a Phenomenon of Metal Metabolism Adaptation in Tumor Cells

Luis Cano
Inserm, Univ Rennes, INRAe, CHU Pontchaillou, UMR 1241 NuMeCan Institute, AEM2 Platform, Rennes

Stéphane Bertani
Université de Toulouse, IRD, UOS, UMR152 Pharmadev, Toulouse

Marie-Laure Island
Inserm, Univ Rennes, INRAe, CHU Pontchaillou, UMR 1241 NuMeCan Institute, AEM2 Platform, Rennes

Juan Pablo Cerapio
Sorbonne Universités, UPMC Univ Paris 06, Institut Pasteur, Unité Organisation Nucléaire et Oncogenèse, Paris

Eloy Ruiz
Instituto Nacional de Enfermedades Neoplásicas, Departamento de cirugía abdominal, Lima

Pascal Pineau
Institut Pasteur, Unité Organisation Nucléaire et Oncogenèse, INSERM U993, Paris

Valérie Monbet
IRMAR, INRIA, Université de Rennes

Karim Boudjema
Service de Chirurgie Hépatobiliaire et Digestive, CHU Rennes, Université de Rennes

Luis Taxa
Instituto Nacional de Enfermedades Neoplásicas, Departamento de Patología, Lima

Sandro Casavilca-Zambrano
Banco de tejidos tumorales - Instituto Nacional de Enfermedades Neoplasicas, Lima

Martine Ropert
Inserm, Univ Rennes, INRAe, CHU Pontchaillou, UMR 1241 NuMeCan Institute, AEM2 Platform, Rennes

Bruno Turlin
Inserm, Univ Rennes, INRAe, CHU Pontchaillou, UMR 1241 NuMeCan Institute, AEM2 Platform, Rennes

Olivier Loréal (✉ olivier.loreal@univ-rennes1.fr)
Inserm, Univ Rennes, INRAe, CHU Pontchaillou, UMR 1241 NuMeCan Institute, AEM2 Platform, Rennes

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Abstract

Our group has previously described a particular form of Hepatocellular carcinoma (HCC) developed in non-cirrhotic liver (HCC-NC) developed in Peruvian patients Our aim is to analyze the HCC-NC from clinical-biological findings in two different cohorts (Peruvian and French) and the link with metallomic profile. Clinical, histopathological and also tumoral (T) and non-tumoral liver (NTL) samples of 38 Peruvian and 45 French patients were studied. Twelve metals were assayed using ICP/MS: Mn, Fe, Cu, Co, Zn, As, Se, Rb, Mo, Cd, Pb, and Sn. Possible associations between metals and survival were also evaluated. Overall, results show clearly differences between both cohorts. Mean age were 41 ± 20 and 68 ± 9 years-old for Peruvians and French, respectively. 80% of Peruvian patients were positive for the HBsAg, versus 9% in French patients. Metals concentrations were higher in the NTL of Peruvians ($p < 0.05$) compared to the French. In both cohort metal concentrations were lower in HCC areas compared to NTL, excepted for Cu for which mean concentration was increased in HCC ($p < 0.05$). Se concentration in HCC was associated with better survival only in Peruvians. Our data shows, in both HCC-NC cohorts, that the process of hepatic tumorigenesis impact similarity the metallomic profile in tumor.

Introduction

HCC is the most frequent primary liver cancer, the sixth most common cancer and the second cause of death by cancer worldwide.[1] Many pathophysiological factors are potentially involved in the onset and progression of HCC, most of them being related to as chronic insult of the liver parenchyma. The development of HCC is commonly regarded a sequential multistep pathogenic process initiated with inflammation-mediated liver tissue damages and hepatocyte necrosis that induce liver fibrogenesis towards cirrhosis, which in turn increases the risk for HCC.[2] Thus, a larger number of HCC cases reported in the literature are found in cirrhotic patients, whereas HCC-NC are more rarely described.

Members of our group previously described a peculiar clinical and molecular presentation of HCC-NC developed by a significant fraction of patients in Peru.[3] These patients exhibited consistently remarkable clinical features: a) 50% of them are relatively young with a median age below 40 years old that includes children, teenagers, and young adults; b) the very large majority of the individuals presented with advanced-stage HCC and tumors larger than 10 cm-diameter; and c) Almost 80% of HCC occurred in non-cirrhotic liver.[4] This peculiar clinical context where HCC-NC affects younger individuals was then corroborated to the whole region of South America.[5]

We further substantiated this peculiar presentation of HCC-NC at both molecular and histological levels. First, Peruvian HCC displayed a unique mutation spectrum, in which the major class of alterations was epitomized by genetic short indels.[6] Second, liver parenchyma exhibited a very low level of inflammatory response and an absence of fibrotic process, and this, despite a strong prevalence of underlying infection with hepatitis B virus.[7,8] However, we observed within the non-tumor liver (NTL) parenchyma the presence of foci of cellular alteration in which cells are smaller compared to regular hepatocytes and exhibit an

altered nuclear-cytoplasmic ratio.[7] These foci of cellular alteration showed also some degree of congruence with the co-expression of precancerous marker glutamine synthetase. Altogether, these findings suggest that the clinical epidemiological situation encountered therein is due to some biological features intrinsic to the natural history of HCC in the population of Peru, and more broadly in South America.[9] This observation prompted us to search for additional pathophysiological cofactors associated with HCC-NC that could enhance the risk of developing at an early age among the Peruvian population.

It has been reported that the toxic effects of metals and their role as cofactor in occurrence of HCC.[10] For example, increased hepatic iron stores has been associated with HCC-NC.[11] Oxidative stress and reactive oxygen species promoted by siderosis are strongly suggested to be instrumental in HCC. Furthermore, As has been classified as an enhancer of oxidative stress and a human carcinogen, notably for HCC.[12] However, other metals such as Mn, Se, and Zn are required for normal activity of antioxidant defense system in cells in a concentration-dependent manner.[13]

In order to evaluate the trace metal levels and whether metals are potential cofactor in the onset of early-age HCC-NC in Peru, we performed a comprehensive analysis of metal concentrations in both HCC and NTL tissues of Peruvian patients (n = 38). In parallel, we contrasted these patients with another cohort of French individuals (n = 45) who developed HCC-NC in utterly different environmental, behavioral, and clinical contexts.

Results

Clinical data and histological data

Table 1 summarizes the clinical and histopathological findings between both cohorts. At time of diagnosis, the mean age of the Peruvian cohort was 40.6 ± 20.1 years-old. A large component of young patients was found in the Peruvian patient group. On the other hand, the mean of age for the French cohort was 67.9 ± 9.3 years old with a unimodal distribution (Fig. 1). A significant difference was found for the age of diagnosis between both cohorts ($p < 0.001$).

Table 1
Clinical and histological variables in both cohorts.

Variable	French cohort	Peruvian cohort	*p*-value
Age (years)			< 0.001
Mean	67.9 (± 9.3)	40.6 (± 20.1)	
Range	[37–85]	[13–94]	
Sex			0.021
M	5	12	
F	40	26	
Tumor size (cm)			< 0.001
Mean	8.7 (± 5.5)	14.3 (± 5.1)	
Range	[1–21]	[4.50–27]	
Hepatitis B virus			< 0.001
Positive	2 (2.8%)	15 (21.1%)	
Negative	38 (53.5%)	16 (22.5%)	
Fibrosis grade			0.145
Absent	5	7	
Grade 1	12	12	
Grade 2	20	8	
Grade 3	8	11	
Grade 4	0	0	
Histological grade			0.010
Well differentiated	9	3	
Moderately differentiated	28	28	
Poorly differentiated	2	7	
Undifferentiated	6	0	
Vascular invasion			0.104
Yes	27	16	

Table footnote: For numerical variables, mean values are presented with ± standard deviation (SD). For categorial variables, data are presented as number of cases. Levels of significance ($p < 0.05$) were calculated with Mann Whitney U-test.

Variable	French cohort	Peruvian cohort	*p*-value
No	18	22	

Table footnote: For numerical variables, mean values are presented with ± standard deviation (SD). For categorial variables, data are presented as number of cases. Levels of significance ($p < 0.05$) were calculated with Mann Whitney U-test.

The Peruvian cohort included 26 men (68.4%) and 12 women (31.6%), whereas in the French cohort, five women (11.1%) and 40 men (88.9%) were found and the difference was significative for gender between both Peruvian and French cohorts ($p = 0.02$). The presence of positive hepatitis B serology (HBsAg+) in Peruvian cohort was reported for 15 patients (48.4%), whereas in the French cohort only two patients (4.4%) were HBsAg+ ($p < 0.001$) (Table 1).

From the pathological point of view, some significant differences were found. For instance, tumor size that was greater in Peruvian cohort ($p < 0.001$). On the other hand, the French cohort presented more cases of undifferentiated HCC compared to the Peruvian one, in which differentiated tumors were largely observed ($p < 0.01$).

Determination of hepatic metal concentrations

Metal concentrations in NTL for both cohorts are reported in Table 2. Among 12 metals evaluated, nine of them including seven essentials (Co, Cu, Mn, Mo, Rb, Se, and Zn) and two toxic (As and Cd) had higher concentrations in NTL of Peruvian patients compared to French ones. Interestingly As was quantifiable only in the Peruvian cohort. To the contrary, Sb concentrations were highest in French patient group compared to Peruvian group.

Table 2
Metals quantification in non tumor tissues between both cohorts.

Metal (ug/gr)	Non-tumoral tissues		*p*-value
	French cohort Mean (± SD) Range	Peruvian cohort Mean (± SD) Range	
Arsenic (As)	0	0.12(± 0.2) [0.00–0.41	< 0.001
Cadmium (Cd)	2.4 (± 2.7) [0–13.3]	4.20 (± 4.1) [0.2–21.3]	0.002
Cobalt (Co)	0.1 (± 0.1) [0–0.3]	0.1 (± 0.1) [0–0.3]	0.05
Copper (Cu)	25.3 (± 31.1) [6.9–204.1]	27.3 (± 12) [9–75]	0.001
Iron (Fe)	873.8 (± 1024.9) [56.2–6275.4]	698.6 (± 721.4) [87–3973]	0.395
Manganese (Mn)	4.6 (± 2.4) [0–12]	7.54 (± 4) [0.9–26.7]	< 0.001
Molybdenum (Mo)	2 (± 1.3) [0–6.5]	3.3 (± 1.5) [0.3–9.4]	< 0.001
Lead (Pb)	0.3 (± 1.2) [0–8]	0.2 (± 0.2) [0–1.3]	0.283
Rubidium (Rb)	17.2 (± 6) [0.9–35.6]	22.15 (± 8.5) [2.4–40.7]	0.002
Selenium (Se)	1.7 (± 0.7) [0–4.1]	2.1 (± 0.8) [0.5–5.4]	0.019
Tin (Sn)	0.3 (± 0.4) [0–1.8]	0.1 (± 0.1) [0–0.5]	0.006

Footnote: Data are presented as mean ± SD and [range]. Levels of significance ($p < 0.05$) were calculated with Mann Whitney U-test.

Metal (ug/gr)	Non-tumoral tissues		*p*-value
	French cohort Mean (± SD) Range	Peruvian cohort Mean (± SD) Range	
Zinc (Zn)	167.6 (± 68.9) [46.2–385.5]	258.1 (± 103.3) [71.9–553.7]	< 0.001

Footnote: Data are presented as mean ± SD and [range]. Levels of significance ($p < 0.05$) were calculated with Mann Whitney U-test.

Metals concentrations in HCC in both cohorts are presented in Table 3. Similarly to what was found in the NTL counterpart, the Peruvian patients had a higher concentration of metals in tumor tissues. As was found again exclusively in the Peruvian cohort, while Pb and Sn concentrations were higher in the French cohort. Concerning Fe, we must point that normal values of this metal are below normal iron concentration in hepatic tissue. Mean iron concentration values were three-fold higher in HCC of French patients (mean = 689.6 ± 1537.9 µg/g) than in Peruvian patients (mean = 255 ± 511.7 µg/g; $p < 0.001$). Remarkably, these concentrations remain within the normal values for normal livers (Fig. 2).

Table 3
Metals quantification in tumoral tissues between both cohorts.

Metal (ug/gr)	Tumoral tissues		*p*-value
	French cohort Mean (± SD) Range	Peruvian cohort Mean (± SD) Range	
Arsenic (As)	0	0.1 (± 0.1) [0–0.5]	< 0.001
Cadmium (Cd)	0.9 (± 2) [0–9.9]	1.1 (± 3) [0–13.1]	0.304
Cobalt (Co)	0.03 (± 0.1) [0–0.4]	0.02 (± 0.1) [0–0.3]	0.157
Copper (Cu)	49.4 (± 87.5) [0–345.2]	65 (± 123.7) [4–543.2]	0.496
Iron (Fe)	689.6 (± 1538) [55.4–9941]	255.9 (± 511.7) [24–3026]	< 0.001
Manganese (Mn)	3 (± 2.9) [0.1–13]	2.6 (± 3.6) [0.1–18.5]	0.140
Molybdenum (Mo)	1 (± 1) [0–4.7]	0.8 (± 1) [0–5.4]	0.121
Lead (Pb)	0.2 (± 0.5) [0–2.7]	0.03 (± 0.05) [0–0.2]	0.840
Rubidium (Rb)	16.6 (± 7) [4.4–42]	16.2 (± 8.8) [2.7–41.8]	0.938
Selenium (Se)	[1.5 (± 0.8) [0–4.5]	1.5 (± 0.7) [0.7–3.9]	0.851
Tin (Sn)	0.1 (± 0.2) [0–1]	0.03 (± 0.1) [0–0.3]	0.152

Footnote: Data are presented as mean ± SD and [range]. Levels of significance ($p < 0.05$) were calculated with Mann Whitney U-test.

Metal (ug/gr)	Tumoral tissues		*p*-value
	French cohort Mean (± SD) Range	Peruvian cohort Mean (± SD) Range	
Zinc (Zn)	99.4 (± 53.9) [17.4–318.9]	104.6 (± 96.3) [23.4–612.1]	0.321

Footnote: Data are presented as mean ± SD and [range]. Levels of significance ($p < 0.05$) were calculated with Mann Whitney U-test.

In both cohorts, we found that metal concentrations were higher in NTL that in HCC (Fig. 3). The exception was Cu that have higher concentrations in HCC that in NTL for the Peruvian cohort. In the French cohort, Cu concentrations were also slightly higher in HCC (49.4 ± 87.5 µg/gr) compared to NTL (25.3 ± 31.1 µg/gr), albeit without reaching the level of significance ($p = 0.22$).

Survival analysis

Seven patients from French cohort were retired from this analysis because they received pre-operatory treatment. Peruvian patients have a survival average of 65.7 weeks; whereas French have survival average of 148.4 weeks ($p = 0.0071$). A multivariate Cox proportional hazard ratio model was developed with all metals concentration in both HCC and NTL. Our results showed that higher Se concentration in HCC samples were a protective feature ($p < 0.004$); while Pb was detrimental ($p = 0.011$), but in the Peruvian cohort only.

Based on the results of the Cox regression model, we performed an analysis to assess the impact of metals on survival. To this aim, we used the values of Se and Pb in HCC and NTL, to create two groups taking the mean concentration value as cut-off, above and below the mean. Our data shows that, the group of Peruvian patients with a Se concentration in tumor greater than 1.49 µg/g had a mean survival of 323 weeks versus 49 weeks for patients below this threshold ($p = 0.033$) (Fig. 4). Such result was not observed in the French cohort.

Discussion

Our study presents, for the first time, a comparative analysis of liver metallomic profiles among patients from different geographical locations and genetic background, affected by HCC-NC, a disease that

escapes the conventional profile of HCC and representing merely 20% of cases of HCC.[14] However in the Peruvian context, HCC-NC represents 90% of cases of liver cancer.[3]

The impact of metals on biological cell processes is a phenomenon not completely understood. Metals are involved in many beneficial functions like maintenance of pH, enzymatic cofactor, metabolism trigger, and reactive oxygen species formation as a product of normal metabolism.[15–17] However, while present in excess, metals may have harmful effects. The principal impact of metals is the disruption of intracellular redox balance, debt to increase of reactive oxigen species production.[18]

Our data shows that concentrations of metals were higher in NTL of Peruvian patients compared to French individuals. Among those metals, some of them, such as As and Cd, are known to exert harmful effects on health.[19,20] Indeed, As and Cd are considered as carcinogenic to human according to WHO. The mechanism by which As contributes to the process of carcinogenesis is DNA damage with chromosomal aberrations, deletion mutations, and aneuploidy.[21,22] A strong link between exposure to arsenic and the development of HCC has been demonstrated in animal models, which also evidenced an increase in lipid peroxidation levels, prior to the onset of the fibrosis process and subsequent development of HCC.[21,23,24] The mechanism of Cd related injury involves the interaction and possible inactivation of thiol groups, leading to functional alteration of the metallo-enzymes of the superoxide dismutase family and to subsequent depletion of antioxidant agents such as glutathione.[25,26]

We cannot assess whether HCC-NC and its spectrum of mutated genes are directly caused by the presence of heavy metals.[27] It is likely that metals could play an enhancing role in the carcinogenic process in association with hepatic carcinogenic agents, such the hepatitis B virus infection in Peruvian patients,[28] and/or alcohol intake that we were not able to assess with sufficient confidence.

However, due to the absence of liver fibrosis in these series of patients, the role of ethanol is predicted to be marginal in the patho-physiological process.

Another important highlight is the common metallome profile of HCC compared to NTL, with lower concentrations of metals in tumor tissue in both cohorts. Such findings suggest that whatever the etiological factors, the geographic origin or other different parameters between cohorts, cancer cells have similar adaptive process regarding metal metabolisms. Whether the concentration decrease of most of these metals are related to a lower uptake, an increase release, and/or an increased turnover related especially to the enhanced cell cycle and cell metabolism remains not known.

The increase of Cu concentration, mostly in cancer tissues, was already reported in HCC,[10] and other cancers affecting especially breast, cervix, ovarian, and lung.[23,29,30] A hypothesis aiming to explain the behavior of Cu in cancer has been proposed by Fisher and collaborators, who states that the increase of this metal is due to a decrease in the catabolism in tumoral cells of ceruloplasmin (Cp) in tumor cells.[31] This phenomenon affecting the multicopper-carrying protein, might be due to increased sialylation produced by free sialic acid from cell membranes of neoplastic cells. This hypothesis was later supported

in an animal model by Bernacki *et al*.[32] Another hypothesis concerns the role of copper as angiogenic agent.[33] McAuslan *et al.* showed that copper acts as promoter of endothelial cells migration.[34] Martin *et al.* consolidated both hypotheses, describing the link between Cu, ceruloplasmin and HIF-1α.[35] The authors showed that Cu acts as a stabilizer of the HIF-1α, mediating inhibition of prolyl-4-hydroylation. The HIF-1α is eventually responsible for regulating transcription of many genes, including the Cp gene. Meanwhile, Himoto proposed a possible mechanism whereby Cu is required for binding HIF-1α to p300 and prevents the effect of Factor Inhibiting HIF-1 (FIH-1).[36]

Another finding is the relationship between Se concentration in tumor tissues and survival in the Peruvian group. Se plays a major role in cell homeostasis, mainly through selenoproteins that are anti-inflammatory, chemo-preventive, and immune modulators.[37] Cox regression model suggests a beneficial effect in overall survival for patients with higher levels of Se in HCC. This result is corroborated by a meta-analysis displaying the negative correlation between Se concentration and HCC.[38] Indeed, low levels of Se have been associated with high risk of developing HCC. Our model developed on a relatively small number of patients give us a significative outcome only in the Peruvian cohort. This could be explained by the fact that, in these patients, a “natural evolution of the disease” is observed,[4] since in this group of patients received only surgery, unlike the French patients who received additional local or systemic treatments that could modify HCC development.[39,40] Such findings shall be corroborated in larger cohorts.

Finally, we must highlight the possible role of environment in the hepatocarcinogenic process due to the high mineral content of the subsoil and rivers. This statment was endorsed by several studies showing the presence of high heavy metal concentrations in the Andean regions of Peru.[41–43] In Egypt, Elwakil *et al.* have also described high concentrations of Cd, Pb, As, and Hg in blood samples from HCC patients who were exposed to the consumption of contaminated plants.[44] Therefore, we cannot rule out the relationship between the presence of metal in environment and natural history HCC.

Altogether, our findings show that Peruvian and French cohorts of HCC patients have different metallomic profiles in NTLs, suggesting a putative impact of environmental and/or genetic factors. Whether these elements play a role in the very peculiar phenotype of HCC in Peru should be further explored. In addition, the modulation of metal concentration in HCC, that is shared by the two cohort suggests a coordinated modulation of metal metabolism in liver cancer cells during the carcinogenesis. Additional studies will allow to progress in understanding the role of metal metabolisms alterations in the hepatocarcinogenic process.

Methods

1. Ethical agreement

The present study investigated in strict accordance with the ethical principles contained in the Declaration of Helsinki. The Peruvian collection was approved by the Human Subjects Committee of the National Cancer Institute of Peru (INEN), Protocol Number INEN 10-05. Written informed consent was provided and signed by participants. When the patient was non-adult, a parent provided the informed consent on his/her behalf. For the French cohort, the biological material used as well as the clinical records data complied with the norms established by the European legislation (2001/20/EC) and the French Ethic Committee.

2. Study design and patient selection

The present study was developed retrospectively in two cohorts, one Peruvian and one French. All patients included in the present study exhibited an HCC-NC, and were treated by surgical hepatic resection. For both cohorts, collected cryopreserved tissues and paraffin embedded tissues of HCC and NTL were evaluated. Bioclinical parameters were collected from clinical records, unfortunately alcohol intake was not extractable from medical records with certainty. Finally, follow-up data for survival analysis were obtained from the National Registry of Identification (RENIEC) in the case of the Peruvian cohort. For French cohort the information was obtained from national obituary system.

Peruvian HCC-NC cohort consisted of 38 patients who were hospitalized at INEN between January 2010 and December 2016. The French cohort was made up of 45 patients hospitalized in the Rennes University hospital between January 2014 and March 2017. Participants from both cohorts were selected on their histopathological report, considering the diagnosis of HCC-NC. For Peruvian cohort, samples were obtained from the INEN Pathology Department and INEN Cancer Research Biobank. For the French cohort, samples were obtained from the Pathology department and Biological Resources Center in Rennes.

3. Histological analysis

Formalin-fixed, paraffin embedded tissues (FFPE) and hematoxylin–eosin (H&E) slides from tumoral and non tumoral areas were stained using the Masson's Trichrome Stain Kit, Artisan™ (Dako), according to the manufacturer's instructions, stain was performed on the Histo-Pathology High Precision (H2P2) platform – ISO 9001 certified – UMS Biosit, within of the University of Rennes, France.

Histopathological analysis of HCC comprises architecture and grading (G1-G4) according to the World Health Organization and the American Joint Committee on Cancer classifications, respectively [45,46]. Liver fibrosis stage (0–4) was then scored in accordance with the scoring system for fibrosis and cirrhosis described by Scheuer and collaborators [47]. All histopathological parameters were independently evaluated by two pathologists (LC and BT). In case of divergence a consensus was adopted.

4. Trace elements quantification

All samples were treated to avoid environmental metal contamination. HCC and NTL samples were desiccated for overnight at 120°C in oven. Then, dried tissues were weighed and mineralized by nitric acid solution in Teflon PFA-lined digestion vessels. Acid digestion was carried out at 180°C using ultrapure concentrated HNO3 (69%) (Fisher Chemical Optima Grade) in microwave oven device (Mars 6, CEM®). The elements studied were either essential - manganese (^{55}Mn), iron (^{56}Fe), copper (^{63}Cu), cobalt (^{59}Co), zinc (^{66}Zn), selenium (^{78}Se), rubidium (^{85}Rb), molybdenum (^{96}Mo), or toxic -arsenic (^{75}As), cadmium (^{112}Cd), tin (^{119}Sn), lead (^{207}Pb). All these were measured by Inductively Coupled Plasma Mass Spectometry (ICP-MS) in a X-Series II from Thermo Scientific® equipped with collision cell technology (Platform AEM2, Biochemical Laboratory, Rennes1 University – Rennes hospital). The source of plasma was argon (purity degree > 99.99%) [48].

The collision/reaction cell used was pressurized with a mixture of helium (93%) and hydrogen (7%); argon and hydrogen were provided by Messer®. Ultra-pure water was provided from Milipore Direct-Q® 3 water station. Nitric acid solution utilized at 69% (Fisher Chemical – Optima Grade®). The rhodium was used like internal standard (Fisher Scientific®). Calibration ranges were realized using a multi-element solution (SCP Science® Plasma Cal). The performance was calibrated using multi-element solutions, tune F and tune A (Thermo®). Certified reference material bovine liver ZC71001 was obtained from NCS Testing Technology (Beijing, China).

5. Statistics

Data collected were inputted into Numbers® software version 5.3 (Apple Corporation). All statistical analyses were performed in R version 3.5.1, "Feather Spray" (R Foundation). The comparative analyzes between both cohorts were performed using the Mann-Whitney test. For survival analysis surgery date was considered as the starting parameter for the calculation of survival. Univariate Cox regression model was done with all variables [49]. Later all variables with significative values were used in a multivariate Cox model, the resulting model was improved with backward and forward stepwise regression. Finally, evaluation between all models was done with Akaike Information Criterion (AIC) index. Best model was selected from lowest AIC value. Finally, for each metal, we used the concentration average for create groups, i.e. over and under the average, and to develop comparative analysis of survival between these groups

6. Data availability

All data generated or analyzed during this study are included in this published articule and its Supplementary information file.

Declarations

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Conlict of interests

The authors declare that they have no competing interests regarding this work.

Author contributions

O.L., B.T., M.R. contributed to conception and design of the study; L.C., J.P.C., S.C., M.L.I., E.R., S.B., L.T., S.C., K.B and M.R. contributed to the data acquisition; L.C., J.P.C, V.M, contributed to the analysis of the data; O.L., B.T., L.C, V.M. contributed to interpretation of the data; L.C., O.L., B.T., S.B.

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Figures

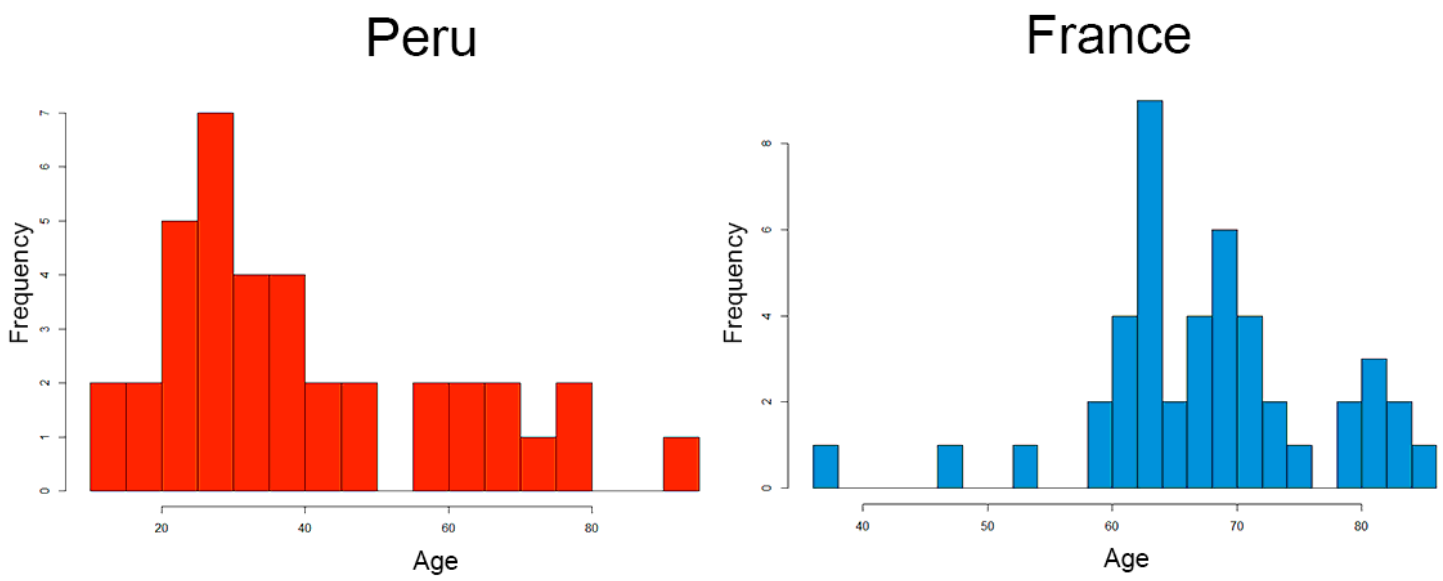

Figure 1

Histogram of age distribution repartition in Peruvian and French cohorts.

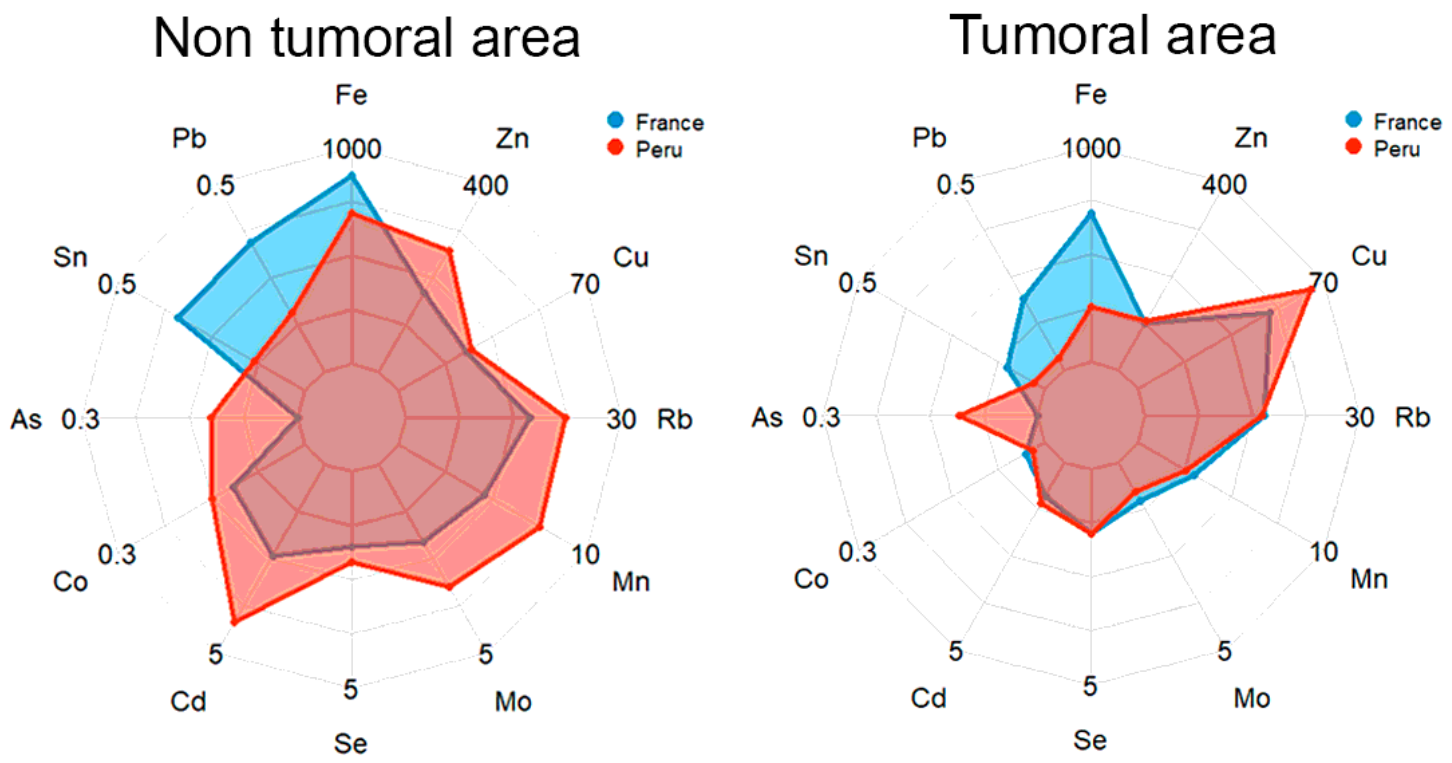

Figure 2

Radar chart for metallomic profile in tumoral and non tumoral tissues. Left panel: Metallomic profile for NTL. Toxic metals as As and Cd are higher in Peruvian cohort (red). However, Fe, Pb, and Sn are higher in French cohort (blue). Right panel: Metallomic profile for HCC. Fe, Pb, and Sn were higher in French cohort. As was exclusively quantifiable in Peruvian cohort.

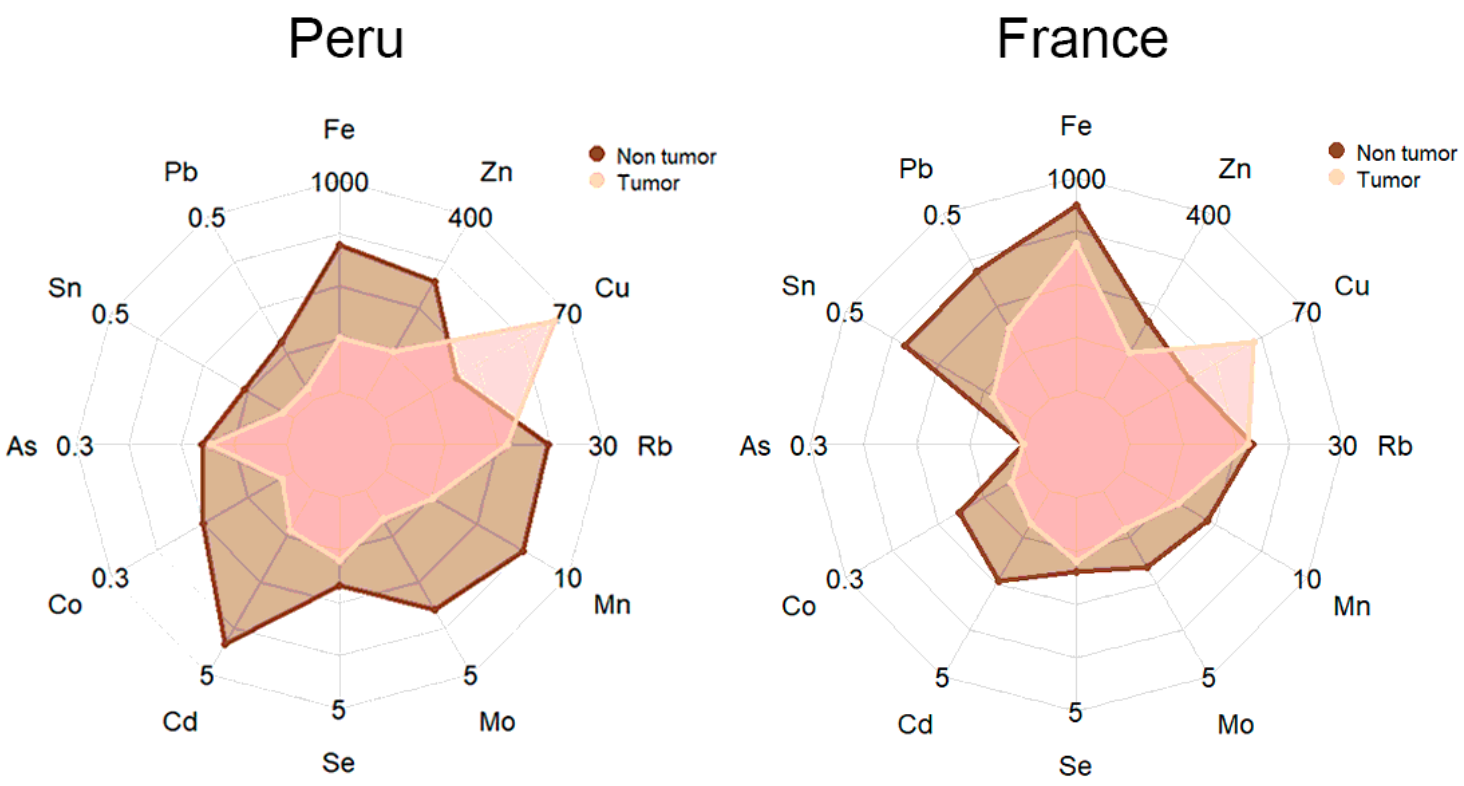

Figure 3

Comparative radar chart for metallomic profile in Peruvian and French cohorts. Radar chart present in every corner a metal and its concentration (µg/g). In French (Left panel) and Peruvians (Right panel), the highest concentrations of metals were found in NTL (darker) except for Cu, which was the only one metal with higher concentrations in HCC (lighter) for both cohorts. As was found exclusively in Peruvian cohort.

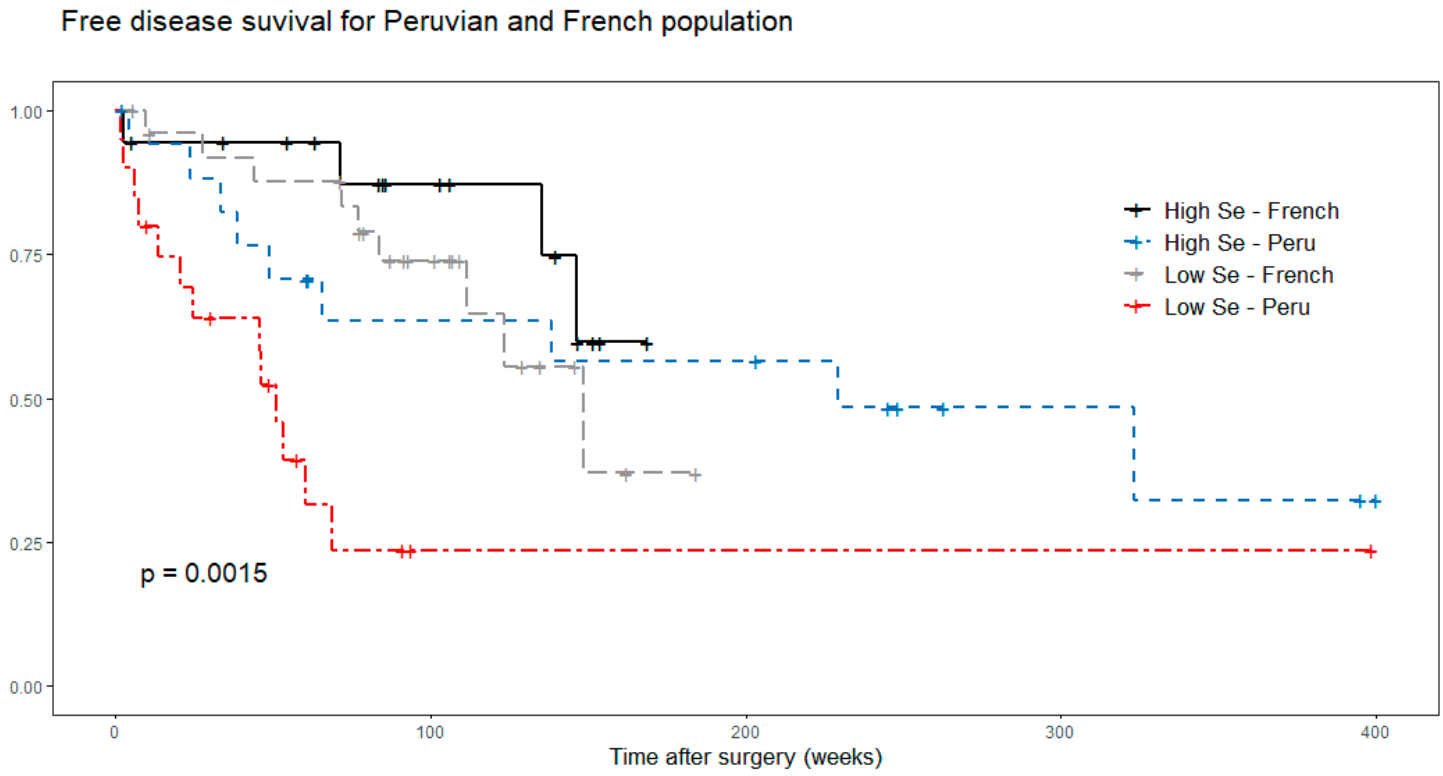

Figure 4

Comparative disease-free survival plot in relation with selenium concentration in tumoral tissues in both cohorts. A significant decrease of survival duration is found for Peruvian patients with low levels of Se in comparison to those ones with higher levels. Level significance ($p < 0.05$) was calculated using Kapplan Meier test.