CHRONIC LOW DOSES RADIATION Why induced reactive oxygen species have a role in cardiovascular diseases ?

DIAPO 2: The question of ionizing radiation impact in cardiovascular diseases (CVD) was in discussion from a long time as a "mysterious" effect or as an artefact effect in medical studies. In this issue data were exclusively epidemiological inquiries at the beginning as usual in radioprotection. Very poor studies were made about direct effects and only for very high exposure on heart (more than 40 Gy). The contradiction between these studies and epidemiological inquiries about low dose exposure, in particular chronicle exposure explained the doubt of UNSCEAR or ICRP.

DIAPO 3: This cohort study based in Canadian national dose registry (NDR) in near 350 000 radiation workers was a good example even if only CVD mortality was explored.

DIAPO 4: The results showed a high significant risk by radiation in CVD mortality **BUT** it was necessary to underline a lack of *non radiation risk factors* impact like : smoking, excessive alcohol consumption, diet, etc… It is difficult in fact to have a comprehensive conclusion out an included second factor studies.

DIAPO 5: The famous "LIFE SPAN STUDY" by THOMSON *et al* in atomic bomb survivors in Japan, underlined for the first time in 2003 CVD mortality in excess (below 4 Gy). LSS study demonstrated that, selection bias, or disease misclassification were excluded. In other hand, it is not a low dose chronic exposure.

DIAPO 6: It is perhaps interesting to have a mention of our French survey in near 2000 nuclear testing veterans with another marker: not the mortality but only the prevalence

compared with "MONA LISA' national study of CVD prevalence in France

DIAPO 7: 82% VETERANS have a NON CANCER DISEASE

DIAPO 8: and it were CVD in 38% with a high frequency than others pathologies.

DIAPO 9: Despite lack inquiry in Chernobyl area, a lot of epidemiological inquiries were done like in workers in British nuclear fuels (Mcgeogghegan 2008) with the same results

1- Low dose chronic exposure have more effects in CVD than short (even intensive) exposure about CVD risks

II- Second factor is needed in any case because the risk in excess was different.

DIAPO 10: Prevalence in veteran French study was slightly but significantly higher than population (38%*vs.* 29.1%)

The same SITUATION for LSS study versus Canadian study: ERR/Sv 0.17 for LLS, **1.35** for NDR **THE QUESTION IS:** why was a difference between studies: is it the question of **CHRONIC EXPOSURE** like in Chernobyl area?

DIAPO 11: The translation from Russian language is difficult, but the idea is that, a direct effect in myocardium cells and, in consequence, ATP activity loss, because a radioactive direct effect IN MITOCHONDRIA? (data, heart rate change in children of GOMEL)

Objections: . if the genesis is a direct heart cell damage, why cardiac disease like cardiomyopathy frequency did not change in all the inquiries? In opposite, why CVD linked with vascular alteration were in excess? And why this effect was not a short term change but a long term effect?

DIAPO 12, **THE CHALLENGE:** What kind of ionising radiation effects could explain this epidemiologic impact in CVD? Why heart, macro and microcirculation were the target? Why it was a long term effect?

Why radio induction was different in short exposure *versus* chronic radioactive exposure? Why second risk factors were crucial ?

DIAPO 13 : **MY HYPOTHESIS :**The radiation effect in this issue is free radicals production; as reactive oxygen species, in blood circulation

The main target is ***vascular endothelium*** and at least, stress oxidative protection loss Chronic endothelium dysfunction is not enough to explain CVD, a second factor is need This mechanism is biologic and not physics.

DIAPO 14 : Free radicals genesis are well known, and some of then are ROS.

ROS are formed by interaction with biological molecules.

ROS attacked molecules, loses its electron and began a chain reaction, and specifically this "chain reaction" have in consequence:

NADPH oxidase in Neutrophil leukocytes became active which converts molecular oxygen to the super oxide anion.

Radiation-induced oxidative stress both in micro and macro vascular endothelial cells, might serve to drive the progression of radio-induced late effects (ROBBINS,2004)

DIAPO 15: Because uncertain measure of blood radioactivity i*n vivo* (up taking and release of radionuclide by cells varies time to time) Experimental study is difficult. only external irradiation is available.

Menandez (2009),Soucy (2007), Collins-underwood (2008) demonstrate that, oxidative stress induced by radiation have a direct action on NO production(protective against oxidative stress), in the delayed phase of radiation.

This effect is also present after low dose exposure

DIAPO 16 : **DISCUSSION I:** ROS production by external radiation and oxidative stress and vascular endothelium

alteration were well demonstrated, but is it the same in human radioactive contamination specifically in a chronic way?
Tribble (1999) demonstrate a crucial role of a second factor (high fat diet) in mice model for endothelial dysfunction. But, again, it was after external irradiation and not in internal radioactive contamination. Is it the same?
We need a *in vivo* model with a chronic oxidative stress, less production of protective vascular endothelium and a second oxidative factor, before any CVD just to understand the relationship between these 2 events.
DIAPO 17, **WHAT WAS THE ANSWER?**
First, an animal model look like the same than chronic oxidative stress and progressive way toward CVD: ROBBINS (2004) demonstrate that, chronic oxidative stress in Diabetes or radiation late effects, *was similar*
Second, In the same model, we need a non radiation risk factor with also an oxidative stress. LYENGAR (1990) and BHAUMIK (1995) demonstrate a generation of free radicals during cold injury and rewarming.
This the reason why *In diabetic rats with chronic oxidative stress, we had experiment cooling and warming up effect as second factor.*
DIAPO 18, Out of to much details The idea for this experiment was that:

- Rats GOTO KAKISAKI (GK) with a very early diabetes *versus* WISTAR rats with normal glycaemia at 4 month age were used. We carry out cooling (from 37° to 32° C) and immediately after warming up (from 32° to 37°C)
- During this time we performed 3 measures of Tc-albumin Trans capillary filtration rate by micro spectrometry

DIAPO 19 , This is the result of oxidation/reduction dosage for oxidative stress level. We can see first, a high and stable level of stress in Diabetic rats from 4 to 5 month age in

agreement with a chronic oxidative stress despite any clinical sign of angiopathy. After 6 cooling /warming up in GK rats, the situation change and we have a very high level of stress more than twice times.

DIAPO 20, The consequences for albumin filtration rate, as a specific marker of micro vascular endothelium function were that:

Before cooling, albumin filtration test was normal even in diabetic rats but not in Diabetic rats after 6 cooling

Cooling have the same effect for all groups of rats, but

After warming up, normal and diabetic rats return to the initial level

IT IS NOT THE CASE FOR THE RAT GROUP AFTER 6 COOLING/WARMING UP. The second factor raised a stable endothelium dysfunction earlier and stronger than diabetic rat group control.

DIAPO 21, Now we could have a credible explanation:

All experimental results might support these mechanisms:

Radiation→freeradicals→oxidativestress→endothelialdysfunction→cardiovascular disease (higher prevalence).

Low dose radiation exposure has effects **long** after exposure, and chronic exposure (like Chernobyl population or nuclear workers) has much more CVD risks than once only exposure (like LSS study)

CVD prevalence is higher and **earlier** if a second factor did exist, like: lipid dysfunction, metabolic diseases, etc…

The second factor have a role not in addition but in **promotion** way as lung radiation exposure and smoking in excess on cancer.

More research is needed, but not as a pretext for delayed decisions by WHO, ICRP, UNSCEAR out in favour of victims.

DIAPO 22, **conclusion**

- **ROS production, oxidative stress and endothelial dysfunction could be a good explanation for CVD in excess after radiation exposure**

- **We need a medical (biological) change for radioprotection, and less physical commitment for exposed population. (risk groups and not only mean dose for a population)**

We need justice for Chernobyl population, nuclear workers and *nuclear testing veterans* and the end of official denied about radio induced CVD.