Appendix

The Impact on Surgical Practice of Recent Advances in Biotechnology. Interactions Between Inherited and Environmental Factors in the Occurrence - and Biological Behavior - of Diseases of Surgical Interest

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The recent advances in biotechnology (molecular biology, genome-wide association studies, proteomics) and in biomedical technology (CT scan, PET, radioimmunoguided procedures, minimally invasive surgery, robot assisted surgery) has dramatically affected the surgeon's decisions, involving both early diagnosis of diseases, multidisciplinary approach, multimodal treatment and also surgical technique and timing of surgical treatment.

Genetics, in addition to provide a precise diagnosis on the basis of the germ-line and somatic mutation, is also useful: (1) to improve our pathophysiologic knowledge; (2) to help us to select the proper treatment and the *timing* for treatment. This is of paramount importance in inherited multitumoral syndromes.

Lesson from Inherited Multitumoral Syndromes

In inherited multitumoral syndromes [1-19], in the presence of an individual with multiple different neoplasms – or at least bound to develop them in the near future – it is crucial to provide a list of the possible neoplasms which are typical of the syndrome and then potentially occurring during the subject's life, along with a scheduled list of treatments, and to select which is the most adequate treatment for that particular subject or when is the best time to do which.

In particular, it must be outlined that within the siblings belonging to a kindred with a given multitumoral syndrome, some neoplasms are *obliged*, i.e. they are bound to occur invariably in all siblings affected by the mutated gene, whereas other neoplasms show variable expression, i.e. they are organic to the syndrome,

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but their actual occurrence depends on interactions between the common germ-line mutation affecting the various siblings and epigenetic changes, which are usually induced by environmental factors [1].

The former group, i.e. obliged tumors, includes colonic polyps and colorectal cancers in patients with familial adenomatous polyposis (FAP) and germ-line mutation of the APC gene, mapped at chromosome 5 q 21, or medullary thyroid carcinoma, occurring invariably before age 5 in subjects with MEN 2A or 2B and germ line mutation in the RET gene, mapped at chromosome 10q 11. In particular, the precise site of the mutation in the RET gene (codon 619, 620, etc.) will dictate the biological behavior of the tumor.

Among the latter, i.e. those occurring only in some of the affected siblings and with a combination varying from individual to individual, we could include, in patients with FAP, papillary carcinoma of the thyroid [3-11] or brain tumors [15-18] such as medulloblastoma, but also desmoids tumors and other extracolonic manifestations, including hepatoblastoma and hepatocellular carcinoma [12-14,] ampulloma or pancreatic and bile tract tumors.

In particular, concerning both thyroid carcinoma and brain tumors, a striking female prevalence has been observed in FAP associated tumors, i.e. a F:M ratio of 50:1 in FAP associated papillary thyroid tumors, during the last 20 years, instead of 3:1 as occurs in sporadic tumors, or 18:0 as in the personal series of FAP PTC reported in Table 1, or a F:M ratio of 4:0, brain tumors (Tab. 2), instead of 1:2 with male prevalence as usually occurs in sporadic medulloblastomas.

Whereas colonic polyps and cancers occur homogenously in males and females [1, 19], this striking female prevalence in some extracolonic manifestations strongly suggests that the germ-line APC mutation plays a facilitating or predisposing role, but other factors, namely environmental factors, and likely factors associated with female sex, could also play an important role.

FAP associated PTCs and brain tumors are neoplasms which are integral to this multitumoral syndrome, because they occur only in patients with the specific APC germ-line mutation (and are more frequently associated with one germ-line mutation instead of another). However, additional factors are required for them to occur (environmental, dietary etc.). In addition they can be considered a typical example of interaction between genetic predisposition and environmental factors. In particular, since FAP-associated PTCs showed an increased incidence 5-10 years after the Chernobyl accident, it is possible that nuclear accidents could also determine long-term consequences, even in subjects living at long distance (thousands of km), in frail or genetically predisposed subjects [11].

Tumors integral to multitumoral syndromes are also likely to have a better biological behavior than their sporadic counterpart.

In particular, duodenal and/or pancreatic endocrine neoplasms associated with MEN1 showed a better survival and a reduced incidence of liver metastases than similar sporadic tumors, whereas breast cancers in subjects with BRAC1 and BRAC2 germ-line mutations were more frequently of the medullary histotypes, which is associated with a better prognosis. It has been suggested that patients with inherited germ-line mutations, facilitating the occurrence of multiple primary

Table 1 A personal series of cases comprising patients with FAP-associated papillary thyroid carcinoma (PTC)

Patient number	Sex	Age	Codon number	Exon number	CHRPE	LOH APC gene activation	Ret/PTC	BT
1	F	30	140	3	-	np	np	-
2	F	19	593	14	+	np	np	-
3	F	22	778	15	+	-	+	+
4	F	31	937	15	+	-	+	-
5	F	18	976	15	+	np	np	-
6	F	27	993	15	+	np	np	-
7	F	39	1105	15	+	np	np	-
8	F	34	1105	15	+	np	np	-
9	F	25	1068	15	+	-	+	-
10	F	26	1061	15	+	-	+	-
11[b]	F	22	1061	15	+	-	+	-
12[b]	F	20	1061	15	+	-	+	-
13[b]	F	36	1061	15	+	-	-	+
14	F	24	1061	15	+	-	-	-
15	F	20	1309	15	+	-	+	-
16	F	27	1309	15	na	np	np	-
17	F	22	na	15	na	np	np	-
18	F	20	na	na	na	np	np	-

APC, adenomatous polyposis coli; *BT,* brain tumor; *CHRP,* congenital hypertrophy of retinal pigment epithelium; *FAP*, familial adenomatous polyposis; *LOH*, loss of heterozygosity; *np*, not performed; *na*, not available

[a]In two patients, also with brain tumors

[b]Hepatoblastoma and hepatocellular carcinoma in a member of this kindred

Table 2 Brain tumors associated with PTC in the same patients or FAP kindred

Study	Sex	Age[a]	APC mutation	Brain tumor histotype	Patient age	PTC	CHRPE
Crail (1994)	M	24	1061	Medulloblastoma	24	+[b]	nr
Lynch (2001)	F	29	1061	Medulloblastoma	30	+[c]	nr
Fenton (2001)	F	29	1061	Medulloblastoma	6	+[b]	+
Plawski (2004)	nr	35	608	Cerebral flax tumor	na	+[c]	nr
	nr	10	608	Brain fibromatosis	10	+[c]	nr
Gadish (2006)	F	21	1061	Pinealoblastoma	18	+[c]	nr
Present series	F	22	778	Craniopharyngioma	16	+[b]	+
	F	36	1061	Medulloblastoma	32	+[c]	+
	F	20	1061	-	-	+[c]	+
	F	22	1061	-	-	+[c]	+

APC, adenomatous polyposis coli; *CHRP*, congenital hypertrophy of retinal pigment epithelium; *FAP*, familial adenomatous polyposis; *na*, not available; *nr*, not reported; *PTC*, papillary thyroid carcinoma

[a]Age (years) of first diagnosis of colonic polyps

[b]In the same patient

[c]In another member of the same kindred

tumors in different organs or districts stimulate a stronger immunological response (lymphocytic tumoral infiltration) which could be responsible for this more favorable biological behavior.

At any rate, it is noteworthy that even within the same kindred with the same germ-line mutation or within the same individual with an inherited multitumoral syndrome, environmental factors are of importance, both due to the occurrence of supplementary tumors, in addition to those *obliged*, i.e. occurring in all the subjects affected by the syndrome, and their aggressiveness.

Surgeons should become familiar with genetics and molecular biology of multitumoral syndromes, either for proper screening and early diagnosis of the various neoplasms occurring in each individual, or his/her siblings, or for the proper treatment and timing of treatment, which usually depend on the type of mutation and the biological behavior of these peculiar tumors.

Lesson from Inherited Multitumoral Syndromes Including Early Aging

It is likely that surgeons in the future will with increasing frequency be faced with individuals showing multiple primary solid tumors in diverse and not related organs and districts, even in the absence of inherited multitumoral syndromes. This is a consequence of the increased life expectancy and prolonged survival after diagnosis and treatment of the first malignancy [1, 11, 20-29].

Immunology and genetics suggest that the occurrence and progression of clinically evident malignancies is strictly related to aging and changes in mechanisms of the immune defense. In particular, some inherited syndromes, such as Werner's Syndrome and Rothmund-Thomson Syndrome, Bloom Syndrome and similar syndromes are characterized by early aging and greater predisposition to the occurrence of malignancies at an earlier age, in comparison with the rest of the population.

Interestingly, it has been suggested that in subjects with germ-line mutations of the genes responsible for these syndromes, environmental factors (air pollution, dietary factors) could determine a greater damage, with more severe complications in these frail predisposed individuals [1, 2, 11, 20-28].

In addition, it is well known that tumors occur or progress more rapidly or have a more severe behavior in immunodeficient or frail subjects. Their occurrence and development points to the break of the balance among noxious agents and host defenses. Subjects become *predisposed*, and it is more frequent and probable that a new tumor occurs in an individual who has previously been affected by other malignancies (Tab. 3).

Both multitumoral syndromes and multiple tumors in the absence of an evident inherited syndrome must be taken into account and could help proper detection of the new tumor. This is of basic importance, because early diagnosis is still one of the main criteria responsible for better treatment and increased survival.

Table 3 Personal series of patients with colorectal cancer associated with multiple different solid tumors in the absence of a detectable inherited multitumoral syndrome. All these patients had no clinical sign of colorectal malignancy. Cancer was detected by colonoscopy because of increased CEA values, after excluding recurrence

Age	Sex	Cancer and surgical procedures	Previous malignancies	Follow up
62	M	Ductal pancreatic carcinoma	Pancreatoduodenectomy	Alive (73y) and disease free (>11y after pancreatectomy)
64		Segment VII liver metastasis	Bisegmentectomy VI-VII	
68		Prostate carcinoma	no surgical treatment	
70		Colorectal (left colic angle) carcinoma	(Colectomy)	
40	F	Ductal breast cancer	Mastectomy	Death at age 68, because of colorectal metastases
45		Thyroid tumor	Thyroidectomy	(>9 years after hepatectomy)
60		Hilar cholangiocarcinoma	Left hepatectomy + CD lobe resection	
66		Colorectal (sigmoid) carcinoma		
49	F	Endometrial carcinoma	Hysterectomy with bilateral adnexectomy	Alive (69y) and disease free (>10y after hepatectomy)
59		Hilar cholangiocarcinoma	Right hepatectomy + CD lobe resection	
64		Right colon cancer	Colectomy	
58	M	Ampulloma	Pancreatoduodenectomy	Alive (69y) and disease free (>11y after pancreatectomy)
64		Left colon cancer	Colectomy	

Lesson from Hepatobiliary and Pancreatic Malignancies

Focusing on some hepatobiliary pancreatic tumors, it is well known that the 5-year survival after radical treatment of ductal carcinoma of the pancreas is only 10% and that in some patients with ductal adenocarcinoma disease free survival could also be greater than 10 years, i.e. the tumor can be considered clinically cured, as in some of our personal cases with pancreatic ductal carcinoma (or hilar cholangiocarcinoma) (See Tab. 3). In contrast, the remaining 90% of pancreatic cancers have a homogeneously dismal prognosis, with an average survival of 10-15 months, however aggressive and potentially radical the treatment. Extended lymphadenectomy and parenchymal resection have been invoked, but overall prognosis has little changed, despite the improvement of clinical skills and technology.

It is likely that among the apparently homogeneous group of subjects with ductal adenocarcinoma there is a great majority of subjects who will have a dismal prognosis, whatever treatment we provide. In contrast, there is a smaller minority that we are not yet able to identify in advance – because the tumor appear histologically similar – who will have long survival after radical treatment (Tab. 3).

The recently extended use of neoadjuvant chemotherapy in the multimodal treatment of pancreatic cancer facilitates the exclusion from radical surgical treatment of subjects with *no response* to preoperative chemotherapy. Even if we do not yet know why these patients do not respond, this preselection actually goes in the right direction of restricting aggressive and demanding surgical treatment only to those subjects with a more favorable biological behavior of the malignancy.

However, we have honestly to admit our current ignorance concerning a crucial issue, i.e. the timely detection of *which is which*, in order to reserve radical treatment and even extended and aggressive procedures to those subjects with more favorable tumors, and to select for palliative or low-risk treatments those with invariably dismal prognosis.

Lesson from Liver Regeneration

A better knowledge of pathophysiologic changes after liver resection has dramatically improved our approach to major liver surgery, in particular in patients with hilar cholangiocarcinoma. These subjects, usually severely jaundiced and septic because of previous endoscopic retrograde cholangiopancreatography (ERCP) [11, 23-26], are extremely frail and with reduced potential for liver regeneration after major liver resection, including more than 60% of liver parenchyma (trisegmentectomy, left or right, always including caudate lobe resection i.e. removal of segments I-VI- or IV-VIII plus I).

Therefore, in addition to procedures aimed at improving biliary stasis and cholangitis [25, 26], preoperative chemoembolization is usually performed of the

liver segments which are planned to be removed. This determines hypertrophy and improved function or residual segments, which could facilitate patient survival during the immediate postoperative period. In fact, after portal chemoembolization, liver volume of residual lobes increases of about 30%, usually within 4-5 weeks [27].

Pathophysiologic and oncological data show that:

- the strongest and most adequate stimulus facilitating liver regeneration and production of cytokines and growth factors is parenchymal trauma to the liver, in particular liver resection;
- liver regeneration, which usually occurs within 30-45 days after resection, involves all residual liver, with a *normal* regenerative response from normal parenchyma, but with tremendous hyperproliferative reaction from residual neoplastic tissue.

Therefore, removal of a single neoplastic nodule or multiple liver metastases, leaving in situ residual neoplastic nodules, must be considered with great caution in the absence of a planned multimodal treatment (e.g. removal of a small liver nodule to perform a correct diagnosis, when the primary tumor is still undetected).

In fact, in the presence of multiple liver nodules, these peculiar pathophysiologic consequences must be adequately taken into account, before planning liver resection, even using *minimally invasive surgical procedures*, if the possible side-effects of the procedure might include the *explosive* growth of residual tumors.

This is a typical example of how recent advances in biotechnology (molecular biology, genetics, a better knowledge of the role of growth factors in liver regeneration) should cope with advances in biomedical technologies (laparoscopic surgery or use of new therapeutic and diagnostic devices) in order to avoid severe side-effects and complications of the incorrect application of new technologies and provide the best therapeutic options for each individual.

Recent advancements in genetics and nanotechnology, in addition to being possible tools for therapeutic purposes, are also useful for a better knowledge of pathologic mechanisms and biological behavior of malignant (and non malignant) diseases.

In particular, in the near future it will be easier to distinguish between subjects with more favorable biological behavior and therefore susceptible to more aggressive and demanding surgical treatment and those who, regardless of an apparent radical surgical treatment, will have a dismal prognosis (e.g. 80-90% of subjects with ductal pancreatic carcinoma) [27].

Better therapeutic results are expected using an integrated multidisciplinary approach, combining both recent advances in biomedical technologies and a better knowledge of basic disciplines, including pathogenetic mechanisms and pathophysiologic linkages.

Lesson from Genetics and Genetic Engineering or Advances in Biotechnology: Surgical Implications

New drugs with molecular target (monoclonal antibodies against EGFR, VEGFR, anti c-kit drugs) are considered with increased interest and have shown to provide significant improvement in the treatment of patients with malignancies. However, even though we are still waiting for the *silver bullet*, i.e. a drug or device which is able to invert a pathologic pathway, and destroy or tackle selectively malignant cells with a low to nil impact on normal cells, it is self-evident that in the present context there is no chance for a single gene therapy, i.e. the possibility that the re-introduction of a wild-type gene in a subject with a mutated gene can switch the damaged pattern on again and enable complete tumor regression. In fact, before a clinically evident tumor occurs, not a single genetic mutation, but a long train of different genetic and biological alterations must develop, usually occurring during a long time lapse. It is therefore not realistic that a single drug or the reintroduction of a normal gene, instead of a previously altered one, or a new product of genetic engineering will be able to invert a long-term altered and chronically impaired mechanism responsible for a clinically evident malignancy.

In contrast, on the one hand while it is likely that new drugs, devices or treatments will be able to co-operate or be used together for a better multitumoral and multidisciplinary treatment of a single patient with single or multiple tumors, on the other improved biotechnology and the development of new devices and instruments or new technological advancements will also improve our present knowledge of basic pathophysiologic pathways and mechanisms which are responsible for the occurrence of malignancies and/or determine their severity or biological behavior.

In fact, thanks to further development in new technologies such as genome-wide arrays, proteomics and metabolomics, it is likely that in the near future we will be able to detect which is which, among various tumors affecting the same organ or district, and showing apparently the same histotype, but having a dramatically different prognosis. In other words, it may become possible to detect in advance which tumors have the best biological behavior, i.e. are less aggressive, more responsive to treatment, and therefore are going to have a better prognosis and yield an increased survival. This knowledge will dramatically affect the surgical approach, indications for surgery, technical procedures and the extension of planned resection, including the selection of a mini-invasive approach or, in contrast, justify an aggressive approach and extended resection, including major vessel resection and reconstruction, because of a *known favorable* biology and biological behavior of the tumor. Or, in case of unfavorable biological behavior, exclude surgical treatment in favor of palliative treatments.

Lesson from Pollution Related Diseases

Air pollution consists of tiny ambient particles measuring < 10–15 micron (PM10) and arising from dust, smoke, or aerosol liquids produced by vehicles, factories, or burning wood. In vitro studies have shown that exposure to diesel soot and other PM10 particles activates pro-inflammatory genes in a process mediated by free radical/oxidative stress mechanisms. These, in turn, induce pro-inflammatory transcription factors, such as nuclear factors-B (NFRB) and activator protein 1 (AP-1), which promote increased histone acetyl transferase activity, histone acetylation, release of interleukin 6 (IL-6) and interleukin-8 (IL-8), markers of inflammation, and, finally, expression of inflammatory genes [28-37].

The adverse health effects of air pollution are difficult to dissect since the atmosphere contains about 18,000 different substances, each of which is present at very low concentrations. Despite the well-known in vitro toxicity, mutagenicity, and carcinogenicity of many pollutants documented by experiments in animal models, it must be stressed that in most of these in vitro studies, the exposure level to each pollutant, e.g. polycyclic aromatic hydrocarbons (PAHs) and TCDD (dioxin), is higher than that occurring under actual conditions, in which PAHs are present at 10 parts per million (ppm), ozone at ppb (parts per billion), and TCDD at ppt (parts per trillion). Therefore the health damage caused by a single pollutant, even after long-term exposure, is likely to be very low.

In particular, airborne pollutants are currently considered weak pollutants. These are responsible for health effects which occur as a no-threshold phenomenon, namely that is no threshold above which all humans are affected and no threshold below which no effect is observable [31-33]. In other words, even very low concentrations of airborne pollutants (PM) can be responsible for health effects in particularly susceptible individuals [31-33].

Although this behavior has been confirmed both by epidemiological and experimental data, a linear dose-effect relation is still considered the main medianistinal linkage between concentration of pollutants and health effects. In other words, the greater the concentration of pollutant, the greater the number of hospital admissions.

In January 2008, a municipal fee (ECOPASS) was introduced in Milan to reduce the entrance of pollutant vehicles within the city. As a result, there has been an evident reduction in the vehicular traffic by up to 30-35% of private vehicles.

A scientific project called Prolife, involving all major local Universities, was started in January 2007, i.e. 1 year before the introduction of Ecopass. The project aimed at analyzing at 360° all the possible implications of health effects of air pollution, with particular reference to host-particle interactions in humans. The first step involved reproducing the same cross-sectional and longitudinal studies in Milan that have been performed in the United States and other countries.

In particular, during 2007-2009 we performed a comparison between the concentration of various pollutants and hospital admissions (53,514 hospital admissions because of cardiovascular and respiratory symptoms), and also longitudinal studies

in small cohorts of children attending primary schools (close to or far from main crossroads) and of old patients living in nursing homes, also comparing those living in hospices close to or far from main crossroads.

In particular, we performed 2 two-week campaigns per year, with mobile monitors placed either indoors and outdoors at the schools and nursing homes (corridors and gardens, respectively) and compared these direct field measurements with clinical findings, in particular in children, who all had a unique referral hospital. This made it possible to analyze any pollution related hospital admission and complications in children enrolled for longitudinal studies [33].

Lastly, the filters obtained during these first-hand seasonal campaigns, during which we measured both qualitative and quantitative parameters and for which we had the comparison with clinical findings, were used for in vitro studies.

Obviously, at the beginning, we reproduced previous in vitro studies using pulmonary alveolar cell lines (A549) or bronchial epithelial cell lines (BEAS-2B). However, after these preliminary studies, we also tried to make the same in vitro studies, with the same types of particles and the same variable concentrations, focusing on 2 distant districts, which on the basis of clinical and epidemiological findings could be affected by air pollution, namely sperm cells and synoviocytes [33-35].

In fact, it has been frequently reported that fertility is decreasing in males living in metropolitan areas, whereas rheumatoid arthritis is increasing, at least in females exposed to increased pollutant concentration, namely to traffic related pollutants.

The main reason for our choice was that both rheumatoid arthritis and male infertility – in particular in patients with varicocele – are two diseases in the occurrence of which autoinflammation plays a crucial role. In particular, the damage to sperm cells can be measured by semiquantitative methods, including both morphologic and functional parameters. This feature enabled us to make a quantitative comparison of the functional effects, not only varying the type and concentration of pollutants, but also varying the type of the host. In fact, we performed 3 different studies, using as host the rabbit, the normal human, and men affected by varicocele [33-35]. Data obtained suggested that, at least for some diseases, the variation of the host could be more important in terms of functional loss than the variation of the concentration or of the type of pollutants.

Lesson from In Vitro Studies of Airborne Pollutants on Synoviocytes and Sperm Cells

Oxidative stress is a working hypothesis that has been suggested as a common mechanistic linkage between particulate material and adverse health effects. But it is not unique. In particular, in a recent in vitro study in which different types of particles were used (PM < 2.5 or ≤ 10 μm/m^3 in aerodynamic diameter, tire debris), the same concentration of different particles with the same exposure time elicited different effects on sperm cell function (motility, viability, rate of apoptosis).

However, variability of the observed effects was less than that elicited by changing the host, with lower adverse effects in New Zealand rabbit sperm [34], more evident effects in human sperm, and very severe effects in humans with previous impaired sperm function (e.g. varicocele) [28]. In particular, sperm function could be reduced by up to 80% of the initial values in sperm cells from some humans with varicocele (unpublished data). Sperm cell function is easy to quantify and compare not only among different pollutants but also among different host species or subgroups [33, 34]. These findings are also in accordance with recent epidemiologic data showing more pronounced respiratory and cardiovascular effects in patients with previous respiratory and cardiovascular impairment or specific susceptibility, respectively [38].

Daily levels of PM_{10}, $PM_{2.5}$ and PM_1 were sampled and various concentration of PM ($10\mu g/cm^2$, $50\mu g/cm^2$, $70\mu g/cm^2$) were incubated at 37°C for 24, 48, 72 hours with synoviocytes from 5 patients with rheumatoid arthritis (RA) and 5 patients with osteoarthrosis (OA).

Synoviocyte-like fibroblasts (SLF) are cells of mesodermal origin that line the synovial cavity and are considered of importance in the pathogenesis of RA.

In particular: (1) PM was engulfed within synoviocytes; (2) after entering the cell, it determined an increased cytokine production (up to 9-fold the basal level of IL6) ($p<0.001$); (3) this increased inflammatory reaction was more pronounced in synoviocytes from subjects with RA than in those from subjects with OA; (4) this 9-fold increase in IL-6 produced by cells which are distant from the portal of entrance of PM – and this is crucial for the occurrence of RA in humans – was greater than similar increases in cytokines, which had previously been observed by our group incubating PM, with cells from front-line districts (alveolar macrophages, A549 alveolar epithelial cell line; bronchial epithelial cell-line BEAS-1B [36].

These data show that SLF are able to determine an autonomous inflammatory response, which greatly also depends on the *intrinsic* properties and genetic imprinting of synoviocytes. Namely, those deriving from subjects with RA seem to be more inflammogenic than SLF from the control group (subjects with OA), thus suggesting that individual susceptibility, i.e. inherited predisposition associated with previous patient history and acquired susceptibility, plays a role greater than intrinsic toxicity of PM in the occurrence of RA [36].

Lesson from Asbestos Exposure and Mesothelioma in Humans

Data in accordance with these observations were also found in couples with the husband, who was occupationally exposed to asbestos for more than 30 years and who had not developed either asbestosis or mesothelioma, and the wife, who was exposed to asbestos only through her husband's clothes, who developed pleural mesothelioma. This lesson from asbestos exposure, together with recent results in patients with mesothelioma from Cappadocia [39, 40], suggests that individual susceptibility is of major important in the occurrence of asbestos related diseases [31-33].

In particular, a genome-wide analysis performed in a small subset of these couples (husband occupationally exposed, but unaffected, and wife unexposed but affected) showed a panel of differently expressed genes which were partly different from couple to couple, and partly common. Interestingly, common genes with diverse copy numbers included major histocompatibility genes, genes involved in the metabolism of xenobiotics and genes involved in the inflammatory response (Tab. 4).

Therefore, a great bulk of clinical and laboratory data suggests that the occurrence of clinically evident diseases is not simply related to the intrinsic toxicity of various pollutants. In fact, if intrinsic toxicity of pollutants were the main cause of health effects, diseases should be similar and homogeneously distributed in the various hosts. On the contrary, it seems that host-particle interactions generate health end-points, which greatly depend not only on individual susceptibility, but also on the type of the response and on the severity of the reaction, which are strictly related to previous patient history or immune habitus (RA vs OA) and also to tissue specificity [33-36].

In particular, we hypothesize that PM-related diseases are not simply determined by an inflammatory mechanism due to intrinsic toxicity of pollutants and mediated by oxidative stress as unique *common mechanism*, but more complex responses are generated, namely those typical of autoinflammatory and/or autoimmune diseases, which are more pronounced or clinically evident in predisposed subjects. And the degree of magnitude of the final clinical outcome can depend – at least for some diseases such as RA – more on host susceptibility and reactivity (host immune response) than on intrinsic toxicity or concentration of PM [33-36]. This will open new avenues for a basic role of autoimmunity in most PM related diseases. In particular, if PM actually reaches the synovial cavity, if autoinflammation is crucial for RA occurrence, and RA is part of the *pollution related syndrome*, then even low local concentration of PM could be sufficient to trigger pathologic events in predisposed individuals.

On the basis of the overall amount of available data we estimate that about 15-20% of the general population can be affected by a whatever disease linked with air pollution, even if susceptibility for each disease may be as low as 1%, whereas some individuals may be susceptible to multiple diseases in different organs or districts. Clinical experience shows that a greater proportion of susceptible children have non-susceptible parents. Therefore, in addition to inherited susceptibility, acquired susceptibility linked to environmental factors, including air pollution, plays an important role in the occurrence of this greater proportion of *susceptible individuals*. It has been proposed that, in particular for the respiratory tract, which reaches its final development after adolescence, exposure to air pollutants during the *perinatal susceptibility window* could be of major importance [36, 37].

There is increased evidence that children whose mothers have been exposed to increased concentrations of air pollutants during the third trimester of gestation or who have been exposed personally during the first months of life have a greater incidence of asthma at age 4 [37], i.e. there is a delayed effect, in comparison with the time of actual exposure. It is presumed that, during this high susceptibility window, even low proportions of pollutants may exert significant damage, i.e. the effect of

Table 4 Allelic imbalance analyzed by comparative genomic hybridization (peripheral blood) of the husband, who was occupationally exposed to asbestos for 30 years, without mesothelioma, and his wife, who was not occupationally exposed (only indirectly exposed through her husband) and who developed pleural mesothelioma at age 72. Nine genetic polymorphisms (7 with involved genes and 2 with no involved gene) were identified. list of genetic alterations (n=7) and involved genes

Mutation	Position	Gene	Protein	Function
1 Del 1q31.3	193513-193614 kB	CFHR1 CFHR4	Complement Factor H-related 1/4	Immunological response and lipid metabolism
2 Dup 4q13.2	69203-69311 kB	UGT 2B17	UDP-glucuronosyl-transferase (UGTs)	Conjugation and elimination of potential toxic xenobiotics
3 Dup 4p15.33	763-873 kB	ZTH HC11	Zinc-finger DHHC domain 11	
4 Del 6p21.32	32595-32660 kB	HLA-DRB5 HLA-DRB1	Major histocompatibility complex class II DR	Immune system regulator, by presenting peptides from extracellular proteins
5 Dup 8p11.22	39341-39449 kB	BCO 67864	AK128178	ADAM5 protein / hypothetical
6 Del 15q12	19869-19988 kB	OR4M2 OR4N4		Genes encoding for olfactory receptors
7 Del 15q14	32482-32751 kB	CR749361	Golgi autoantigen golgin-67	Golgi apparatus maintenance

this perinatal exposure can be amplified by one or more degrees of magnitude in comparison with later exposure of the same subject. Experimental studies also seem to support this view. In addition, since damage from xenobiotics during the perinatal period affects epigenetic mechanisms, previously unsusceptible subjects may become susceptible, and this acquired susceptibility may also be transmitted to future generations [37].

This mechanism, together with other mechanisms on pathogenic linkages, could be relevant for a better knowledge of how inherited factors (such as genetic alterations or germ-line mutations) may interact with environmental factors (diet, pollution, radiation), generating effects which could be restricted to the subject of interest, but which could also determine a permanent change in the genome or epigenome which is transmissible to offspring and which could be responsible for the occurrence of chronic or malignant diseases.

In particular, during the perinatal susceptible window weak pollutants or dietary toxicants which usually do not elicit severe or permanent alterations because of their very low concentrations could determine effects similar to those of in vitro studies that are usually performed with *pathologically relevant* concentrations, which are some degrees of magnitude greater than real world exposure. These mechanisms and these interactions should be better known not only by epidemiologists, statisticians and scientists involved in correlation studies, but also by surgeons, to be used in their everyday clinical practice.

In other words, a large proportion of the most critical effects in humans could occur because of exposure to increased pollution during perinatal life. The effects of this exposure may become evident many years later, as asthma at age 4, or in adult life as COPD or as tumors or cardiovascular diseases (or rheumatoid arthritis) in the elderly. Therefore, there will be an asynchronism between the time of exposure and the occurrence of clinically evident effects [36].

It may be possible, although this requires further documentation, that the greater bulk of future long-term effects (i.e. those related with acquired susceptibility) is actually due to exposure to pollutants during the first years of life. According to this hypothesis, even *pollution related lung cancer* (there are different types of lung cancers differently linked with smoke and/or air pollution) could be mainly determined during these first years, i.e. the exposure during these crucial years could play a greater role for the occurrence of future diseases than future exposure during later life.

These observations and inferences based on different pathophysiologic mechanisms greatly affect the results of epidemiologic studies comparing pollutant concentrations and concomitant hospital admissions. In fact, the latter are going to completely miss the health effects in newborns, which could be comparatively the most important [30-36].

Our working hypothesis [32, 33, 35, 36] is the following: usual concentrations of pollutants in most Western countries is responsible for very few and non-severe health effects in healthy humans. In contrast, the same pollutant concentrations could determine significant and severe effects in the elderly, or in children, or in frail predisposed individuals. Even among children and old people, clinical out-

comes will be evident only in a minority of the subpopulation. At the moment we are unable to distinguish which is which. A critical exposure will be possible in the perinatal period, during which the effect of pollutants could be amplified, and effects could be determined either in previously susceptible or in previously unsusceptible individuals, which will turn into susceptible because of this perinatal exposure. This is a crucial aspect of our hypothesis, which could explain the increased incidence of susceptible people during the last decades including those affected by nonmalignant diseases such as asthma, BPCO, cardiovascular diseases or male infertility, but also pollution associated malignancies [35, 36] (Figs. 1, 2).

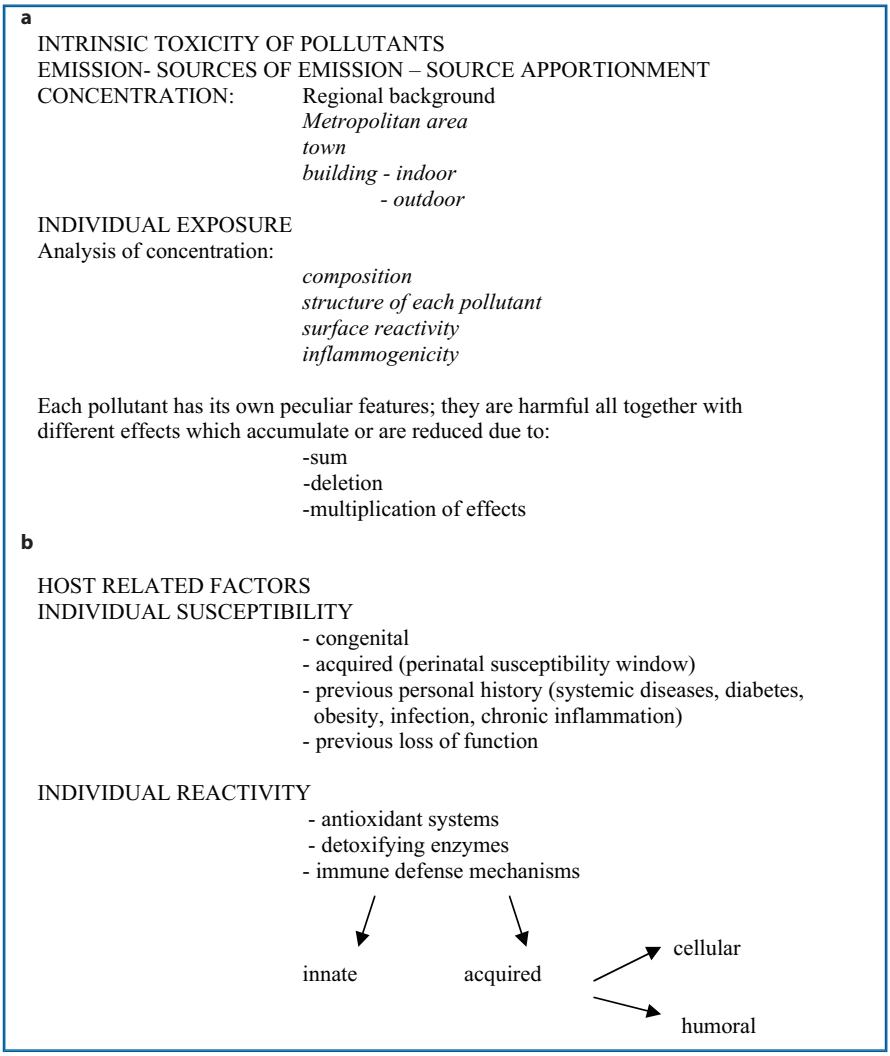

Fig. 1 Host-particle interaction depends on the particle side (concentration, composition, structure, surface reactivity of pollutants) (**a**) and on the host-side (individual susceptibility and reactivity) (**b**)

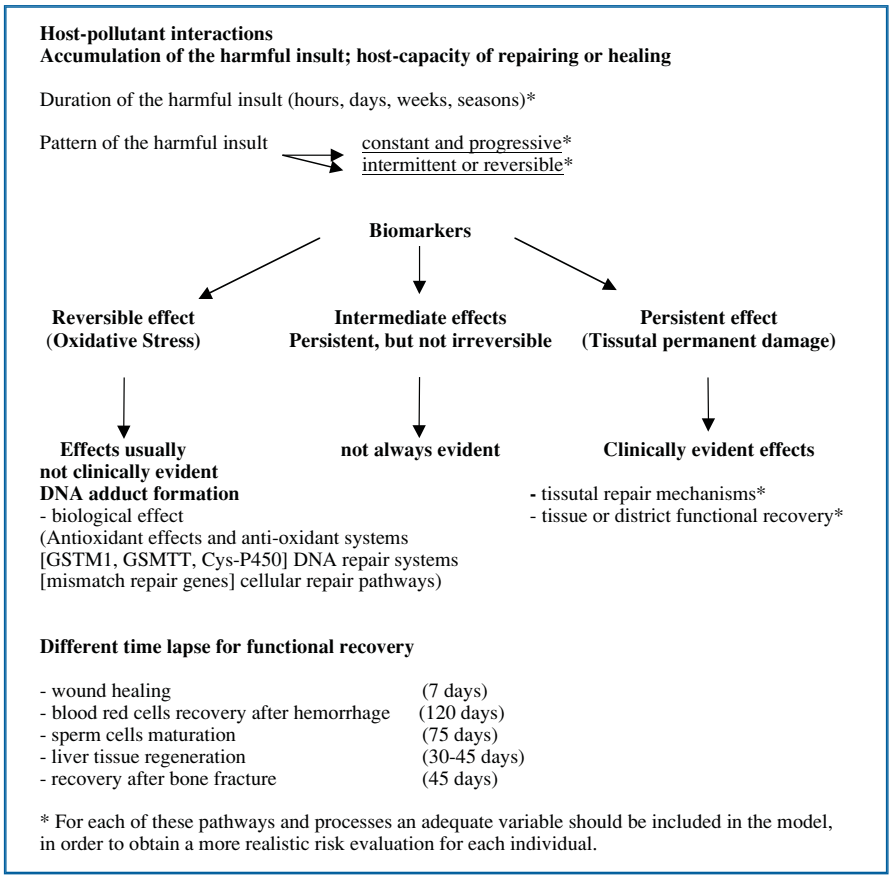

Fig. 2 Clinically relevant outcomes of host – particle interactions do not depend only on intrinsic toxicity of pollutants, but also on host- reactivity and repair ability of the host, which includes gene repair and cell function repair, but also tissue repair and functional recovery. Accordingly, biomarkers will include: markers of oxidative stress (reversible), intermediate effects (persistent) and of permanent tissue damage (irreversible). Only the last markers of irreversible effect are strictly related to final outcomes. The absence of reliable markers of persistent effect of PM is one possible factor of the lack of correlation between pollutant concentration and diseases responsible for hospital admission

Since individual susceptibility (or resistance) is crucial for the future occurrence of clinically evident diseases or symptoms, it is presumed that not a *pure toxic* mechanism will be responsible for clinical outcomes, i.e. a causative mechanism determining severe effects which are homogenously distributed in all individuals on the basis of the concentration of pollutants (linear dose-effect relation), but a different mechanism, involving more host related factors will be responsible for most final health effects (Fig. 3).

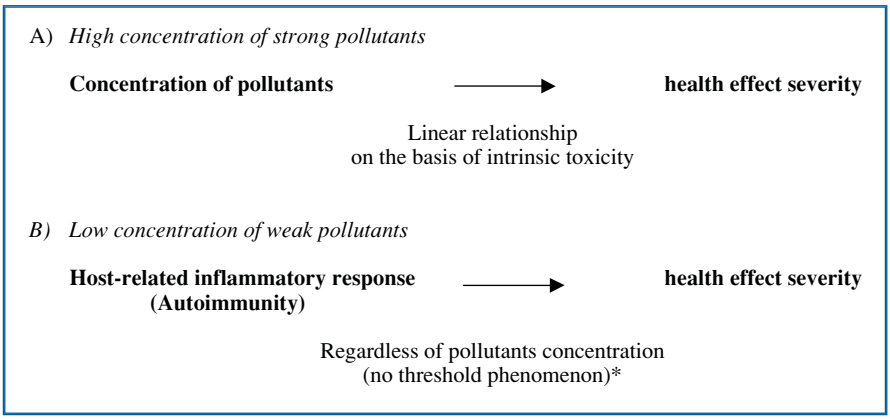

Fig. 3 Current paradigms to explain health effects of air pollution are mainly based on a linear dose and effect relationship (the greater the concentration of pollutant the greater the effect). An alternative pathogenic mechanism suggests that, at low concentration of weak pollutants, health effects could be due to host-related autoimmune mechanisms, relatively independent of pollutant concentration

Suggestions to Policy Makers to Tackle Health Effects from Air Pollution

If most pollution-related clinically evident diseases occur because of a major role of inherited or acquired factors (because of previous personal history or previous exposure), it may be more logical and more affordable as an objective to allocate resources into early recognition of susceptible at-risk individuals than to try to abate further PM concentration. The latter in densely populated metropolitan areas may appear as a non realistic goal, and also extremely expensive in terms of cost-benefits for human health.

Therefore, if the main goal of antipollution measures is not the simple reduction of measured air parameters, but a better treatment and/or a proper prevention of pollution related clinically evident diseases in humans, instead of fixing anachronistic and often difficult to reach objectives in metropolitan areas, where presence of humans and related activities (population density) are the most relevant causes of air pollution, a *sound* objective to reach could be the early detection of individuals at greater risk because of increased susceptibility, in order to electively address proper therapeutic and prophylactic measures for this subpopulation of at-risk subjects.

Lesson from Epidemiologic and Genome-wide Association Studies

Recently proper attention has been drawn to the rhetoric of false-positive results concerning environmental determinants and human health outcomes. In particular,

Boffetta et al. [41, 42] suggested that "users of epidemiological results outside the scientific community ... should be aware of the fact that statistically significant or positive results are often false" and that "epidemiology is particularly prone to the generation of false-positive results." In particular, in a review of 39 highly cited (citation index >1.000) randomized controlled trials that reported an original claim of an effect [43], only the results of 19 trials were replicated by subsequent studies. Therefore, caution should be applied in the communication of results to the media and the general public, because both tend to consider numbers and percentages as the *truth* and make their own speculations on data that are often based on inferences and weak associations [41].

However, the question of non-reproducibility of scientific results cannot be reduced to a mere controversy among epidemiologists – a controversy that should be limited strictly to them and treated only by improving statistical methods. It actually affects the basis of empirical knowledge, in particular when it involves biological and medical questions. When sensational new discoveries are counter to empirical observation, caution is mandatory. Biases may be detectable by epidemiologists, but there are other possible sources of basic errors concerning pathophysiologic mechanisms that are peculiar to each disease and that are unknown to statisticians, who apply the same methods to a wide variety of different conditions [2].

Biological plausibility is not enough. Individual susceptibility plays a role greater than previously supposed in the occurrence of clinical outcomes in the host due to environmental factors. The importance of susceptibility reflects a decreased relative role of pollutant concentration (i.e. intrinsic toxicity of xenobiotics [inhaled or ingested]), and reduces the applicability of certain models – based on dose and effect linearity – to no-threshold phenomena [2, 31-34] (Fig. 3).

Proper selection of subgroups, which should be homogeneous not only for age and sex but also for pathophysiologic relevance, is not only an epidemiologist's task but should be directed also by clinicians and pathologists. For example, lung cancer is still considered by epidemiologists as a single entity, but clinicians are aware that, in addition to cancer occurring in an anthracotic lung, pulmonary cancers may also occur in non-anthracotic lungs; this is a different disease less likely to be dependent on air pollution [2]. The knowledge of this fact will greatly affect population selection.

In particular, a senior clinician (surgeon, internist, clinical specialist) having long-term experience with the disease of concern should always be involved in the design of the study and in reporting study results. Interdisciplinary control of research is not only a desirable option, but a necessary measure to mitigate the sensational effect of new discoveries. This is true in particular when, despite statistical significance of observed differences, findings are counter to everyday clinical experience, or they are not clearly adherent to – or a logical consequence of – strict criteria such as Koch's postulates [1, 2]. Clinicians could also suggest the proper timing for large and expensive epidemiological trials, which should be performed exclusively when adequate metrics and reliable pathophysiologic causative mechanisms between determinants and outcomes have been established. Our view is that clinicians should be involved both in study design and timing, so that interdisciplinary control of the study can be guaranteed from the beginning [2].

Conclusions

The recent advance in biotechnology has dramatically changed not only the surgeon's approach to diagnosis and treatment of diseases of interest, but also his role in multidisciplinary research.

Concerning malignant diseases, the increased life expectancy that has been achieved with dramatic improvements in the diagnosis and treatment of primary tumors has been accompanied by the occurrence of second or third solid tumors in some cancer patients. These multiple tumors are apparently not related to germline mutations of tumor suppressor genes [1].

Increased exposure to traffic-related air pollution in densely populated metropolitan areas and to a wide variety of genotoxic xenobiotics introduced either by diet or by inhalation, together with spontaneous mutations related to aging are likely responsible not only for the observed incidence of chronic inflammatory diseases but also of malignant tumors. Future research will better elucidate the mutual, highly complex interconnections between inherited and environmental factors in the occurrence of malignancies. The phenotypic manifestations of the same germline mutation of a tumor suppressor gene are highly variable, even when patients belong to the same kindred. This is mainly due to superimposed epigenetic factors, which could be sex-based or environmentally related [1]. Likewise, health damage from occupational exposure to known carcinogens such as PAHs or even asbestos greatly varies among individuals with the same exposure level and/or belonging to the same family because of individual susceptibility [32-37, 44-46]. This includes inherited predisposition due to ethnic or individual differences not only in genetic polymorphisms for the genes encoding enzymes involved in xenobiotic metabolism, but also in *acquired predisposition*, related to the effects of aging, concomitant chronic or metabolic disease, such as infections, immunodepression or diabetes, and variable exposure to environmental agents, beginning from fetal development and/or the first weeks of life.

On the one hand, surgeons should be more prone to learning lessons from other disciplines, namely oncology, genetics, molecular biology, biotechnology and bioengineering in order to use these new technologies not only for better knowledge of the biological behavior of the various diseases, but also for the proper treatment and a multidisciplinary approach to new complex phenomena (for instance, multitumoral syndromes, or multiple primary tumors or metachronous tumors). On the other hand, surgeons, as well as all clinicians belonging to various subspecialties, should be involved from the beginning in the design of epidemiologic or correlation studies, which up to now have been considered the exclusive realm of geneticians, biologists, molecular biologists, epidemiologists, statisticians, concerning the degree of association between congenital factors or environmental factors (air pollution, dietary habits) and clinically evident diseases, including a better knowledge of mechanistic linkages and causative mechanisms. In fact, the latter should not only be biologically plausible on the basis of in vitro studies and studies in experimental animals, but should also be relevant, from a pathophysiologic point of view,

to clinical pictures and diseases observable in the real world. Otherwise, improper inferences can be made by epidemiologists or statisticians or biologists, who try to make uncorrected extrapolations from their data [41-43].

Therefore the surgeons and clinicians of the future, on the one hand should be more specialized in a smaller subset of their own discipline to guarantee state of the art treatment to their patients, while on the other they should be open to cooperate with other specialists, and play a primary role from the beginning in the design of epidemiological studies concerning the health effects or clinical outcomes of inherited germ-line mutations or environmental factors (such as air pollution, dietary habits), or aging or age related alterations, in order to avoid biased results and improper inferences, which otherwise are very frequent (up to 85%) [41-43] in the absence of deep involvement of clinicians in multidisciplinary studies.

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