Scientific scope of integrating research activities in the Janus Serum Bank and Cancer Registry of Norway

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BACKGROUND

In recent years there has been an increased interest in using biorepositories in cancer epidemiology to investigate etiological factors as well as define biomarkers of early detection, treatment and prognosis. Advancing cancer research through biospecimen science requires a high focus on quality (1,2). When introducing molecular epidemiology in the 1980s, the expectation was that it would help overcome some major limitations of epidemiology and facilitate cancer prevention. This first generation of markers has contributed to the understanding of risk and susceptibility related largely to genotoxic carcinogens. Today a number of new and promising biomarkers are becoming available for epidemiological studies, thanks to the development of high-throughput technologies and theoretical advances in biology (3). One major challenge in biobank-based research is the access to large series of high quality biospecimens and data on disease outcome and confounding variables.

The Janus Serum Bank was established in the early 1970s with the aim of searching in the premorbid sera for chemical, biochemical, immunological, or other changes that might indicate cancer development at early stages, or be indicative of increased risk of cancer (4-6). It is a population-based biobank with blood serum samples from 317 000 individuals from 17 counties in Norway. The main part of the collection derives from screening health examination participants (90%) and the remaining 10% is from Red Cross blood donors in Oslo. The Norwegian Cancer Society financed the operation of the bank up to 2004, since then it has been fully integrated within the Cancer Registry of Norway. This integration optimizes the use of the biobank by providing the unique opportunity to contribute with biological specimens and high quality clinical data from cancer patients. The development, sample collection, quality assurance and methodological considerations in Janus projects are described elsewhere (7). In the present communication we want to describe the experiences from 35 years of operation of a prospective cancer biobank.

QUALITY ASPECTS IN BIOBANKING

Good biobanking practice requires a quality manual containing Standard Operational Procedures (SOPs) to ensure professional handling at all levels of operation. The aim of a quality manual is to specify routines and methods for storage and use of the specimens that will give the best scientific return within the available budget (8). To meet these requirements a Janus quality manual was compiled about ten years ago, as part of participation in the EU 6th Framework Network of excellence *"Cancer Control using Population-based Registries and Biobanks"*. The manual includes SOPs of information security, data management, handling of samples, project logistics and deviation i.e., content overview is available at www.kreftregisteret.no/janus. Validation of an established SOP is important to ensure that the proposed procedure fits the purpose. The SOPs in Janus are in accordance with NCI, OECD and ISBER guidelines (9-11).

The sample collecting and handling in the Janus Serum Bank has been fairly unchanged over time (7). The effects due to preanalytical sample handling, storage temperature (–25 °C) and long storage time of the samples, have been measured in stability studies of electrolytes, metabolites, enzymes, immunoglobulins, transport proteins, vitamins and hormones. Bilirubin (light sensitive) and potassium (sensitive for prolonged clot-time) were influenced by the sample handling (12). Sodium indicated about 4% sublimation in the samples after 25 years storage. The studies further showed small or no changes for serum level of calcium, iron, creatinine, uric acid, albumin, AST, cystatin C, IgE, IgG, SHBG and transferrin after 25 years of storage. However, ferritin, ALT, CK (12,13) and folate (14) were subjected for degradation or changed conformation.

Although the biobank was not established to collect genetic material, the samples contain trace amounts of DNA that enable molecular analyses within limited scope. So far the samples have been used in two genotyping studies with a success rate of up to 97.7% (15,16). The novel biomarker microRNA has recently been found to be remarkably stable in long-term stored samples from the Janus repository (17). Furthermore, a number of ongoing research projects will provide answers regarding the utility of the DNA material in the various specimens.

A historical biobank as Janus will reflect time specific changes in the background population and differences in serum components between old and new samples may therefore be caused by other factors than storage conditions. For instance, relatively large

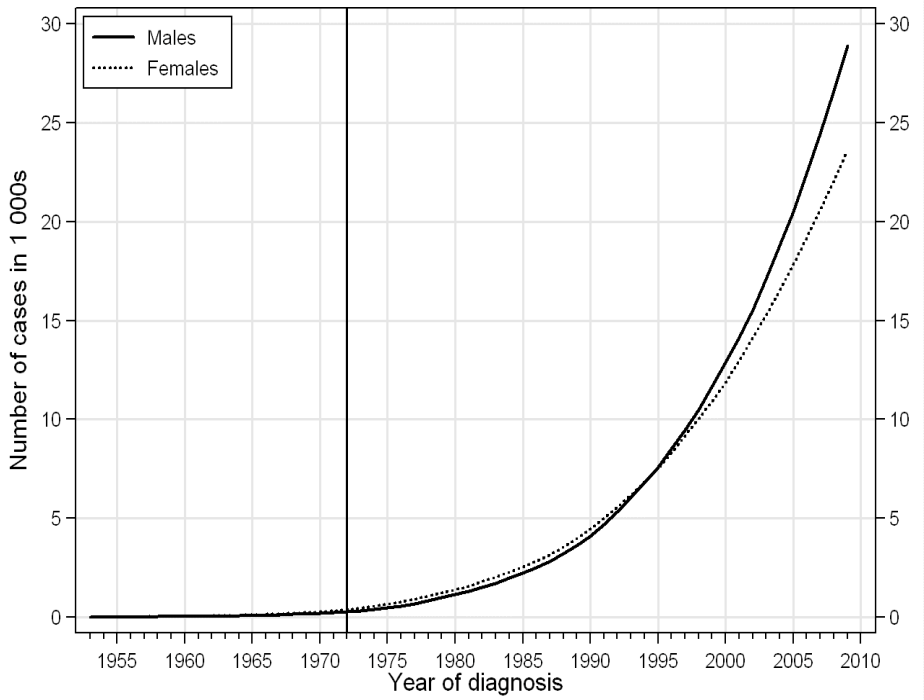

Figure 1. Number of cancer cases among Janus donors in the period 1953-2009 by year of diagnosis and sex.

changes in life style have taken place in the Norwegian population the last decades, including increased obesity, increased alcohol use, less smoking and less physical activity, that all may influence biochemical processes. This is an important aspect to consider in the interpretation of analytical results, in studies comparing samples over calendar time.

An important hallmark in the Janus Serum Bank is the unique code-keeping policy in research projects. The code of the case-control status is never revealed before the laboratory analysis is finalized, and results are returned to the Janus secretariat for long-term archiving. This procedure protects against manipulation of results and research fraud. In collaboration with Nordic biobanks we have recently published a paper focusing on important quality aspects in biobank based research, like study design, methodological approaches, matching variables, selection criteria, data linkages and logistics (18).

CANCER INCIDENCE

Once a year, the Janus cohort is linked to the Cancer Registry to identify new cancer cases among the donors. The cohort members were followed up for the event of cancer in the period 1953 to the end of 2009. The number of cancer cases has increased year by year since 1978, the largest increase in the period 1995-2009 (figure 1). The number is expected to increase in the years to come as a consequence of the aging Janus cohort.

By the end of 2009 the total number of incident cancer cases with remaining blood volume in the Janus repository was 52 464. Table 1 shows the sub site specific numbers of cancer in the period 1953-2009. As shown in the table a considerable number of rare cancer sites have developed among Janus donors, for instance 179 cases of liver cancer and 660 multiple myeloma cases.

In male donors prostate cancer is the most common cancer (n=7468), with a proportion of 26% of the total number of cancer cases (figure 2). The second most common cancer is lung cancer (n=3222), followed by colon (n=2205), bladder (n=2040) and melanoma of the skin (n=1663).

Breast cancer is the most common cancer in female donors (n=6774), constituting 29% of the total number of cancer (figure 2). Cancer of the colon (n=1707) is the second most common cancer followed by lung cancer (n=1650) and melanoma of the skin (n=1423). Cancer of the genital organs, cervix uteri, corpus uteri and ovaries combined, counts 4176 cases.

RESEARCH ACTIVITIES AND SCIENTIFIC OUTCOME

Biospecimens from the Janus repository have been utilized in a large number of studies published in international journals since the 1980ies, either in joint collaborative Nordic or European networks or as sole contributor. A complete list of publications is available at the website www.kreftregisteret.no/janus.

The scientific outcome fits well with the intended purposes. In brief; the studies have focused on i) infections and cancer, ii) biomarkers for early detection of cancer, iii) environmental exposures and cancer, iv) lifestyle related risk factors and cancer. As early as in 1997 the association between HPV and cervical cancer

Table 1. Number of incident cancer cases in male and female donors. Follow up 1953-2009.

ICD 10	Site	Males	Females	Total
C00-96	**All sites**	**28894**	**23570**	**52464**
C00-14	**Mouth, pharynx**	**731**	**281**	**1012**
C00	Lip	169	70	239
C01-02	Tongue	150	56	206
C03-06	Mouth, other	142	42	184
C07-08	Salivary glands	56	50	106
C09-14	Pharynx	214	63	277
C15-26	**Digestive organs**	**6121**	**3874**	**9995**
C15	Oesophagus	301	74	375
C16	Stomach	892	370	1262
C17	Small intestine	113	77	190
C18	Colon	2205	1707	3912
C19-21	Rectum, rectosigmoid, anus	1577	954	2531
C22	Liver	131	48	179
C23-24	Gallbladder, bile ducts	126	132	258
C25	Pancreas	698	459	1157
C26	Other digestive organs	78	53	131
C30-34, C38	**Respiratory organs**	**3567**	**1734**	**5301**
C30-31	Nose, sinuses	62	27	89
C32	Larynx, epiglottis	256	45	301
C33-34	Lung, trachea	3222	1650	4872
C38	Mediastinum, pleura (non-mesothelioma)	27	12	39
C40-41	**Bone**	**42**	**44**	**86**
C43	**Melanoma of the skin**	**1663**	**1423**	**3086**
C44	**Skin, non-melanoma**	**983**	**478**	**1461**
C45	**Mesothelioma**	**127**	**17**	**144**
C46	**Kaposi's sarcoma**	**9**	**3**	**12**
C47	**Autonomic nervous system**	**16**	**10**	**26**
C48-49	**Soft tissues**	**134**	**169**	**303**
C50	**Breast**	**34**	**6774**	**6808**
C51-58	**Female genital organs**	**-**	**4176**	**4176**
C53	Cervix uteri	-	1025	1025
C54	Corpus uteri	-	1614	1614
C55	Uterus, other	-	12	12
C56	Ovary	-	1282	1282
C51-52, C57	Other female genital	-	13	13
C58	Placenta	-	230	230
C60-63	**Male genital organs**	**8050**	**-**	**8050**
C61	Prostate	7468	-	7468
C62	Testis	479	-	479
C60, C63	Other male genital	103	-	103
C64-68	**Urinary organs**	**3003**	**965**	**3968**
C64	Kidney excl. renal pelvis	866	407	1273
C65	Renal pelvis	97	49	146
C66-68	Bladder, ureter, urethra	2040	509	2549
C69	**Eye**	92	**78**	**170**
C70-72, D42-43	**Central nervous system**	**947**	**989**	**1936**
C73	**Thyroid gland**	**207**	**513**	**720**
C37, C74-75	**Other endocrine glands**	**234**	**186**	**420**
C39, C76, C80	**Other or unspecified**	**459**	**349**	**808**
C81-96, D45-47	**Lymphoid and haematopoietic tissue**	**2475**	**1507**	**3982**
C81	Hodgkin lymphoma	140	108	248
C82-85, C96	Non-Hodgkin lymphoma	1104	712	1816
C88	Malignant immunoproliferative diseases	61	20	81
C90	Multiple myeloma	426	234	660
C91-95, D45-47	Leukaemia	744	433	1177

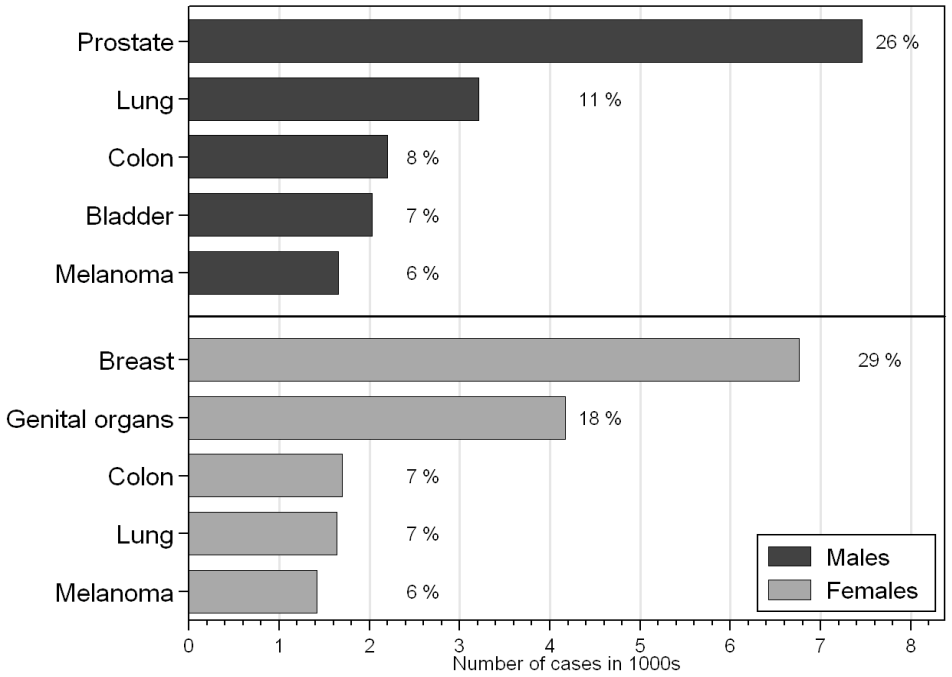

Figure 2. Proportion of common cancer cases among male and female Janus donors.

was found (19). Later, HPV was found to be positively associated with other types of cancers i.e. non-cervical anogential (20) esophageal (21) and head and neck (22). Another early observation from Janus was that Helicobacter pylori could cause gastric cancer (23).

Access to biological samples combined with data from population-based cancer registries and census occupational data, in studies of work-related and environmental exposure, is needed (24). Such studies are possible to perform by using specimens and data from the Janus Serum Bank. Research on biomarkers of environmental exposures is an on-going research topic, e.g. studies on organochlorine exposures and cancer has been performed in collaboration with the National Cancer Institute USA (25-27).

In recent years the applications for access to samples have been more oriented towards molecular epidemiological studies, microRNA profiling in cancer and omics-based studies. There is also an increasing interest in networking projects for data harmonization and sharing. Most recently the Janus cohort was involved in a large consortium of agricultural cohorts (28). A growing trend is also seen in the optimalization of biobanking by reuse of analytical data, published years ago, in new meta-analyses (29).

REMARKS AND FUTURE PERSPECTIVES

The Janus Serum Bank has been and will continue to be an important contributor to international research on biobanking and cancer research. Looking back in time the mode of operation is, in many respects, an example for high quality biobanking. Solutions for several of the current problems were, with incredible foresight, established decades ago. The large number of publications in high impact scientific journals was achieved thanks to efforts of the long-standing highly qualified Janus steering board, which has regulated the access to biospecimens in all research projects. A scientific steering board is indeed recommended and should be mandatory in all biobank operations.

The repository comprises samples stored over a long period of time and presents the opportunity to investigate accumulated, prospectively occurred cancer cases. The sample accessibility to a large number of healthy control individuals make it very timely to set up large studies with adequate statistical power to demonstrate contrasts in exposure between cancer cases and healthy controls, and to address the long-term effects of past exposure profiles, individual susceptibility and time-lag effects.

An important future aim is to communicate the documentation of specimen quality for improved study quality when using samples from an historical biobank. To achieve this a number of stability studies will be performed using samples from different periods of the collection, in the same way as in modern biobank operation (30,31). Of specific interest is the investigation of the quality and amount of DNA-material in the Janus repository. Further, the intention is to utilise modern technology to enable the use of the specimens in gene-environment-interaction and omics-based studies.

The most important future goal for the Janus Serum Bank is to promote excellence in the science of biobanking, ensuring that the repository is optimally used for its designed purpose – contribute to progress in cancer research.

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