Invited Commentary

Invited Commentary: Circulating Inflammation Markers and Cancer Risk—Implications for Epidemiologic Studies

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Chronic inflammation, an established risk factor for cardiovascular disease, is increasingly being recognized as an etiologic factor in several cancers. In this issue of the Journal, Touvier et al. (Am J Epidemiol. 2013;177(1):3–13) report on the association of 7 markers of inflammation, adiposity, and endothelial function with risk of overall cancer and breast and prostate cancers in a nested case-control study carried out within the SU.VI.MAX cohort (France, 1994–2007). Consistent with previous reports on this topic, Touvier et al. focused on a limited number of markers. Future studies of inflammation and cancer should be able to capitalize on emerging multiplexed methods for the simultaneous detection of larger numbers of inflammatory markers in low-volume specimens. This should allow a more comprehensive evaluation of the role of inflammation in cancer development. In this commentary, the authors review emerging methods for measurement of multiplexed inflammation markers, the design and analytic implications of the use of these methods in epidemiologic studies, and potential public health implications of such studies. Given that many large prospective cohort studies have already collected and banked serum/plasma samples, rapid gains in our understanding of chronic inflammation and its role in cancer etiology are possible.

cardiovascular disease; circulating markers; inflammation; multiplexed assays; neoplasms; reproducibility

Abbreviations: CRP, C-reactive protein; CVD, cardiovascular disease; GWAS, genome-wide association studies; ICC, intraclass correlation coefficient; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants.

Chronic inflammation has long been believed to play an etiologic role in tumorigenesis, but supporting molecular, clinical, and epidemiologic evidence has only recently begun to accumulate (1). Molecular studies show that inflammation and altered immunity play a critical role in several stages in the carcinogenic process, including tumor initiation, promotion, progression, metastasis, and response to therapy (1–3). Likewise, evidence from clinical and epidemiologic studies implicates chronic inflammation and inflammatory conditions arising from infections and other environmental exposures in the etiology of several cancers (Table 1) (4–13).

In this issue of the Journal, Touvier et al. (14) report on the association of circulating prediagnostic levels of 7 markers of inflammation (C-reactive protein (CRP)), adiposity (leptin and adiponectin), and endothelial function (E-selectin, soluble intracellular adhesion molecule 1, soluble vascular adhesion molecule 1, and monocyte chemoattractant protein 1) with risk of overall cancer and risks of breast and prostate cancer in a nested case-control study conducted within the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) cohort (France, 1994–2007). Elevated levels of CRP were associated with increased prostate cancer risk, and elevated levels of soluble intracellular adhesion molecule 1 were associated with increased breast cancer risk (14). Several aspects of the study by Touvier et al. suggest a true association between levels of the circulating inflammation markers they examined and cancer risk. The inflammatory factors in their study are known to act upon pathways relevant to carcinogenesis; thus, the relation is biologically plausible. The markers have high reproducibility, and plasma samples
were collected several years prior to cancer diagnosis, thus ensuring against reverse causality. Touvier et al. also included careful matching for important confounders (e.g., age, sex, body mass index); the cases and matched controls were appropriately sorted in batches; and laboratory analyses were performed in a blinded fashion.

These results add to the growing body of evidence indicating an important role for inflammation in cancer etiology. The field of inflammation research is changing rapidly given recent advances in methods of measuring inflammation markers, which will have important design and analytic implications. Here we review emerging methods for measurement of multiplexed inflammation markers, design and analytic implications of the use of these methods in epidemiologic studies, and potential public health implications of such studies.

Emerging technologies for measurement of circulating inflammation markers

Similar to the study by Touvier et al., most prior epidemiologic investigations of circulating inflammation markers and cancer risk have focused on a limited number of markers, such as CRP, interleukin-6, interleukin-8, and tumor necrosis factor $\alpha$ (10, 15). However, the process of inflammation is complex, involves several cellular components of the innate (e.g., neutrophils, macrophages, mast cells) and adaptive (B and T lymphocytes) immune response, and is orchestrated by several key mediators, such as chemokines, pro- and antiinflammatory cytokines, growth and angiogenesis factors, markers of adiposity and endothelial function, and metabolic markers (1, 2). Therefore, a precise characterization of inflammatory biomarkers and pathways involved in carcinogenesis requires a more comprehensive evaluation of a wide range of inflammatory markers. The measurement of a narrow range of markers in prior studies was driven, in part, by the lack of availability of reliable assays. Traditionally, levels of inflammation markers in serum/plasma samples have been measured using marker-specific enzyme-linked immunosorbent assay methods, which involve significant investments of time and large specimen volumes (16).

The recent advent of multiplexed methods (such as xMAP bead technology (Luminex Corporation, Austin, Texas)) for measuring inflammation markers now allows for the simultaneous measurement of a larger number of analytes in relatively low-volume specimens (16). Notably, methodological evaluations of multiplexed assays show that a high number of analytes can be measured reproducibly, with low within- and across-batch coefficients of variation (coefficients of variation of 10%–15%, which reflect high test-retest reproducibility) and high intraclass correlation coefficients (ICCs) (ICCs >70%–80%, which reflect higher across-person variability than within-person variability), thus highlighting the utility of multiplexed inflammation marker assays for epidemiologic investigations (17–19).

**Epidemiologic implications of the use of multiplexed inflammation marker methods**

While multiplexed measurement of inflammation markers affords the opportunity to comprehensively evaluate the association of several components/markers/pathways of the inflammation process with cancer risk, the use of this technology for large-scale, population-based investigations has key implications for the design, analysis, and replication of epidemiologic studies. Indeed, problems with several of these epidemiologic features have hampered the recent use of similar technologies, such as proteomics, gene expression arrays, and mass spectrometry, for cancer biomarker discovery (20–23).

Future studies will need to be sufficiently large to ensure adequate statistical power and reduce the likelihood of false-negative results, particularly in view of risk estimates ranging from 1.5 to 3.0 in prior studies. Although these risk estimates are generally larger than those in genetic studies, stronger associations would be anticipated under the assumption that inflammation plays a key role in the etiology of several cancers. One reason for the moderate effect sizes is that, while chronic inflammation may play a primary role for some cancers (e.g., gastric cancer), it may act as a cofactor for others (e.g., smoking-associated cancers). Additionally, these estimates need to be interpreted within the context of matching/adjustment for key risk factors associated with both inflammation and cancer, such as smoking.

Intraindividual variability in levels of inflammation markers over time could also lead to attenuation of observed risk estimates. In most studies, inflammation marker levels at a single point in time have been utilized as surrogates for persistent/chronic elevations in marker levels (i.e., chronic inflammation). However, barring a few exceptions such as CRP, the temporal stability of a vast majority of markers is not known. Temporal instability of markers can lead to measurement error, reflected as low long-term ICCs, which can in turn bias risk estimates towards the null (17, 18). Figure 1 illustrates the impact of long-term ICCs (based on serial samples from the same individual, collected at least 1 year apart) on odds ratios. As shown in the figure, even relatively robust ICCs can result in marked attenuation of risk estimates. Therefore, epidemiologic investigators will need to consider multiple measurements over time to accurately identify persons with chronic inflammation.

Table 1. Inflammatory Conditions Associated With Cancer

<table>
<thead>
<tr>
<th>Inflammatory Condition</th>
<th>Cancer Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid reflux disease</td>
<td>Esophagus</td>
</tr>
<tr>
<td>Cholangitis and gallstones</td>
<td>Bile duct/gallbladder</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Stomach</td>
</tr>
<tr>
<td>Hepatitis B and C</td>
<td>Liver</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Colon</td>
</tr>
<tr>
<td>Tobacco smoke and particulate matter</td>
<td>Lung</td>
</tr>
<tr>
<td>Prostatitis</td>
<td>Prostate</td>
</tr>
</tbody>
</table>

**Inflammation Markers and Cancer Risk**

Levels of inflammation markers in serum and plasma samples are sensitive to method of collection, processing, and storage, highlighting the need for standardized procedures in epidemiologic investigations (17, 19). Studies utilizing multiplexed inflammation marker measurements will need to incorporate stringent laboratory testing methods, such as blinded testing of cases and controls in the same analytic batch and assessments of within-batch and across-batch reproducibility of marker measurements through blinded duplicate testing.

Similar to any molecular epidemiologic study, investigations utilizing multiplexed inflammation markers will need to incorporate rigorous epidemiologic design, including the use of prediagnostic specimens to guard against reverse causality and control for key confounders known to be associated with changes in inflammation marker levels. It is generally accepted that despite the added complexity of matching on follow-up time, nested case-control designs are preferable over case-cohort designs for studies involving measurement of biomarkers (such as inflammation markers) that are sensitive to analytic batch variability, storage time, and freezing-thawing (24). This was not done by Touvier et al., as controls were selected from persons who had complete follow-up and were alive at the end of the study (14), potentially resulting in an artificially “healthy” control group. Some of the controls lost to follow-up—such as participants who died from cardiovascular disease (CVD) during follow-up—may have had higher levels of inflammatory factors. Excluding these participants from control selection could have resulted in apparently low levels of inflammatory factors among controls, thus inflating risk estimates. Fortunately, overall mortality and loss to follow-up were low in the SU.VI.MAX cohort, so major bias due to loss to follow-up is unlikely.

Inflammation markers are known to be correlated because of functional redundancy (19). Given the measurement of a limited number of markers, Touvier et al. analyzed independent associations of markers with cancer risk through simultaneous adjustment for all markers in regression models. The measurement of a larger number of analytes would mandate the additional use of more sophisticated analytic techniques, such as principal components analysis or factor analysis, to reduce the dimensionality of data (25). These analytic methods will probably provide insight into the correlation structures across inflammation markers and into key inflammation pathways involved in the carcinogenic process (19). The measurement of multiple analytes simultaneously will also mandate systematic corrections for multiple statistical testing to reduce false-positive associations (26, 27).

In future studies utilizing multiplexed marker measurements, investigators will need to carefully plan replication efforts. Along with adjustment for multiple comparisons within individual studies, this will help limit the number of false-positive findings. In this regard, the lessons learned from past failures of candidate-gene approaches and the
accumulating successes of genome-wide association studies (GWAS) can provide a template for replication of results (28). Heterogeneous case and control definitions, limited study sizes, and lack of standardized statistical analyses and reporting all contributed to the large number of false-positive leads from past candidate-gene approaches to discovering genetic determinants of cancer. On the other hand, stringent planning of discovery and replication phases at the onset, standardized statistical analysis, and careful control for false-positives associations have all contributed to the accumulating success of GWAS (28). Compared with GWAS, the number of exposures under investigation for multiplexed inflammation marker measurements is relatively minimal, the exposures are correlated, and the priors for associations are perhaps stronger. Further, unlike GWAS, multiplexed inflammation marker methods are less amenable to pooling efforts given potential incompatibility of noncontemporaneously tested measurements. Nevertheless, we draw the analogy of multiplexed inflammation marker measurements to GWAS to highlight that a standardized approach to study design, laboratory and statistical analysis, and reporting of results akin to GWAS can greatly enable discovery and replication of key inflammatory markers and pathways involved in carcinogenesis (29).

**What the future might hold: example from CVD**

The evolution of knowledge regarding the role of chronic inflammation in CVD provides the clearest example of the epidemiologic and public health potential of investigations on inflammation and inflammatory markers in chronic disease research (30, 31). Numerous large-scale, population-based prospective studies have addressed the association of CVD outcomes with levels of several circulating inflammation markers: acute-phase proteins such as CRP, serum amyloid A, and fibrinogen; pro-inflammatory cytokines such as interleukin-6; adhesion molecules such as E-selectin, intracellular adhesion molecule 1, and vascular adhesion molecule 1; and generic markers of inflammation such as white blood cell count and erythrocyte sedimentation rate (30–35). Of these, CRP has been the most frequently investigated marker owing to several promising features, such as the availability of reliable assays and the temporal stability of 1- or 2-time measurements in predicting chronic inflammation (30, 31). These studies show that, even after adjustment for standard risk factors considered in the Framingham Heart Study algorithm (age, total cholesterol, high density lipoprotein cholesterol, body mass index, hypertension, smoking, and family history), elevated circulating levels of CRP are associated with a 2.0- to 4.0-fold increased risk of CVD among both men and women, as well as among apparently healthy persons and persons with existing CVD (30–35).

Given these consistent associations, CRP levels are now included in global risk prediction models for CVD (31). For example, the Reynolds risk score for prediction of 10-year CVD risk among men and women incorporates CRP levels and family history in addition to traditional Framingham covariates (36, 37). Validation studies of the Reynolds risk score show that a large proportion of persons originally classified as having intermediate risk can be reclassified as low- or high-risk on the basis of CRP levels (36, 37). Finally, although the precise mechanisms need to be elucidated, recent studies point to the utility of CRP levels in therapeutic decisions for primary prevention of CVD through the use of lipid-lowering statins, which also have antiinflammatory (and CRP-lowering) effects (38, 39).

In summary, investigations of the role of chronic inflammation in CVD have led to significant insights into etiologic mechanisms, risk prediction/stratification, and therapeutic decisions for primary prevention.

Can studies of chronic inflammation in cancer epidemiology lead to similar advances in etiology, risk stratification, and therapeutic intervention? Admittedly, epidemiologic research on inflammatory markers and cancer risk has lagged behind CVD research by several years. Therefore, in the near future, studies will need to focus on establishing etiologic associations. Several key questions will need to be addressed. Do circulating levels of inflammation markers have tissue specificity (i.e., do circulating levels accurately reflect ongoing local tissue inflammation)? Can a 1-time measurement of circulating levels accurately identify persons with chronic inflammation? As these questions are addressed and inflammation markers significantly associated with cancer risk are identified, researchers will need to formally evaluate the utility of markers for disease risk stratification by taking into account the strength of associations as well as performance characteristics, such as sensitivity/specificity and positive/negative predictive values. They will also need to investigate whether markers of inflammation, such as CRP, interleukin-6, etc., are active participants in the etiologic process of carcinogenesis or merely markers of subclinical disease. This question is particularly relevant for understanding the utility of inflammation markers for risk prediction versus therapeutic intervention targeted towards lowering marker levels. While risk prediction is possible for both etiologic markers and disease markers, therapeutic intervention for risk reduction through lowering of marker levels is possible only for etiologic markers.

The availability of several large prospective cohort studies with standardized collection and storage of serum/plasma samples and the availability of emerging technologies to reliably measure a large number of inflammation markers both point to the potential for gaining a greater understanding of the role of chronic inflammation in cancer etiology in the near future.

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REFERENCES


