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A single dose of ketorolac to prevent cancer recurrence after surgery: Something for nothing ?

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A single dose of ketorolac during surgery may suppress cancer relapse: something for nothing?

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INTRODUCTION

Is there a rationale for an anaesthesiologist's role against cancer recurrence?

Despite all the improvements in cancer patients' care, a significant part of these suffer from early postoperative recurrence. This is linked to a high mortality rate and remains a public health problem.

Databases analyses confirm that there is a peak of relapse months after the surgery. The time of this peak seems to be related to surgery, whereas the height of the peak (i.e. the risk of relapse) depends on non-surgical factors, including systemic inflammation.

As a consequence, there is a strong rationale to hope to improve patients' outcome by non-surgical perioperative interventions, especially on host's inflammatory response.

We describe, in this first part, why and how we have planned to study the promising role of the non-steroidal anti-inflammatory drug ketorolac.

The following sections detail our studies and confirm the potential interest of ketorolac, a widely used, non-toxic and cheap analgesic, to prevent many postoperative cancer recurrences.

Is there a rationale for an anesthesiologist's role against cancer recurrence?

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SUMMARY

Growth of tumors can accelerate during the peri-operative period. Accordingly, early relapse of cancer occurs in some patients during the first two postoperative years. Temporal and biologic analyses of cancer pathophysiology suggest a link between peri-operative pathophysiological changes and acceleration of tumor growth. Understanding the complex role of inflammation and its consequences, positive and deleterious (i.e., immune response, growth factors, dissemination of tumor cells), could lead to define a role of anesthesiologists in reducing cancer recurrence following surgery. We argue for peri-operative pharmacological interventions to reduce cancer relapse, with a focus on non-steroidal anti-inflammatory drugs.

1. Introduction

Tumor growth is non-linear, exhibiting alternating periods of dormancy and accelerated growth (1). This growth pattern is changed by surgery. Indeed, analysis of hazard rate of recurrence following resection of a primary tumor has shown an early peak during the first two postoperative years followed by a later peak. The latter represents the progression of initially dormant micro-metastases and does not seem to be due to a direct induction by primary surgery (2,3,4,5). Among possible mechanisms of this early peak, some components of the inflammatory reaction triggered by surgery are thought to play a major role. Inflammation accelerates the growth rate of tumor cells and modifies the equilibrium between proliferation and elimination. Inflammation influences cancer cell dedifferentiation and dissemination, and could also inhibit anticancer immunity (6,7,8).

Anesthetic techniques and peri-operatively administered drugs can affect postoperative inflammation and, consequently, the associated immune dysfunction. This provides a rationale for supporting a role of the anesthesiologist to fight against postoperative cancer recurrence, as suggested in several retrospective studies (3,9,10,11,12,13,14,15,16). Among these interventions, non-steroidal anti-inflammatory drugs may be of particular interest, and need to be investigated in that respect.

2. Increased relapse rate for breast cancer early after primary tumor surgery

2.1. Clinical observation

Over a decade ago, data from the Milan National Cancer Institute suggested that the relapse frequency over time in early stage breast cancer patients treated only by mastectomy could be bimodal (17). Analysis of these data showed an early peak of relapses at 18 months, a nadir at 50 months, and a broader second peak at 60 months, followed by a plateau lasting for more than 15 years (Figure 1). Most of relapses (50-80%) occurred during the first peak. This early postoperative peak was also observed by other investigators in large series of patients, regardless of the administration of any adjuvant therapy (18,19,20). In some cases, detection of this early peak requires the use of smoothed hazard plots (20). The hazard rate of early recurrence is greater in high risk patients. Risk depends on primary tumor size, grade, lymph node involvement, and estrogen receptor status (20,21). Demicheli *et al* also reported a double peak of recurrence in operated lung cancer patients (22). For other cancers, data of sufficient quality are lacking. As a consequence, this pattern of recurrence may be delayed, modified or even absent in some cancers. For example, it seems to be completely different in prostate cancer patients (5). Regarding breast cancer, for which this pattern was confirmed, two possible mechanisms can explain the high incidence of early relapse after surgery: surgery-induced escape from dormancy and surgery-induced dissemination of tumor cells. Both might be affected by anesthesia and analgesia.

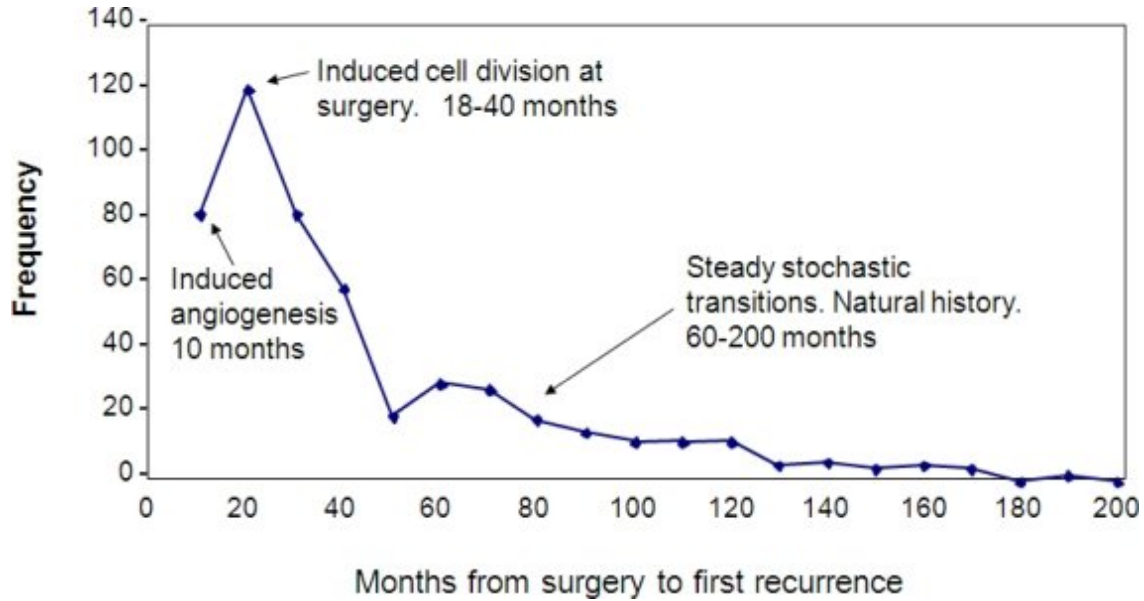


Figure 1. Data from Milan are shown in raw form as number of relapse events in 10-month wide bins. Also indicated are the various modes of relapse that are predicted by the computer simulation. From (17), with permission.

2.2. Surgery and escape from dormancy

The concept of dormancy of tumor cells is not new. It corresponds to a growth arrest and prolonged survival of disseminated single or small groups of tumor cells, and/or to a constant balance between apoptosis and proliferation, keeping the lesion small and undiagnosed (23).

There is a consensus on the existence, in several cancers, of this dormant state of tumors before initiation of overt metastases (20,21,22,23,24,25,26). The escape from dormancy probably involves cell genotype (type of DNA mutation) (1), tumor phenotype (down-regulation of angiogenesis inhibitors and up-regulation of angiogenesis promoters) and tumor environment (including growth factors, inflammatory mediators, and immune cells) (8).

Nonlinear tumor growth has been confirmed clinically in breast cancer. On a series of local recurrences following mastectomy, Demicheli *et al* compared the measured diameters of local recurring tumors after mastectomy for breast cancer, with the calculated diameters under the assumption of continuous growth. They noted that their clinical data were violating this assumption, and this confirmed that growth was non-linear (25).

Escape from dormancy explains other observations in breast cancer patients. These include a greater clinical efficacy of adjuvant chemotherapy in premenopausal node positive women (at higher risk of accelerated growth and early relapses), and the greater efficacy of mammographic screening (to diagnose cancer) for women aged between 50 and 59 years than for those aged between 40 and 49 (17). Indeed, these data show that pathophysiology could

depend on age, and suggest that surgery may destabilize an equilibrium existing between tumor growth and host defenses, particularly in younger women.

The question then arises to know whether surgery is able to induce an escape from dormancy. Epidemiological arguments suggest such an effect in humans, supported by observations in animal models. In humans, the analysis of the efficacy of mammographic screening allowed determining the influence of surgery on the tumor growth rate. Indeed, the timing of the early peak of postoperative recurrences appears to be more influenced by the time of surgery than by the stage of the disease (20). The timings of recurrences are super-imposable, whatever the metastatic sites (viscera, bones, or soft tissues) (4,21,26). The time to recurrence and the time to death may be influenced by the time of surgery. An initial excessive mortality has been suspected in women aged between 40 and 49 years, when surgery was proposed earlier and following mammographic screening (27). Taken together, these data suggest that surgery can promote growth of metastases. However, it might be possible to interfere with this postoperative accelerated growth of residual tumor. In the randomized clinical trial ATAC, the aromatase inhibitor anastrozole reduced early relapses in postmenopausal patients with hormone receptor positive breast cancer, as compared to tamoxifen (5). The peaks of recurrences were still present, but delayed.

In animal models, several results support an impact of surgery on the biology of tumor cells. In rats, we and others have observed surgery-induced tumor progression of metastases of a syngeneic mammary adenocarcinoma (28,29,30). A similar influence of surgery has been observed in other cancers. Through an analysis of growth rate of liver metastases in patients with colorectal carcinomas, Peeters *et al* have reported a similar observation, showing that tumor growth rate is significantly higher after resection of the primary tumor (31). This suggests that surgery can modulate tumor growth by decreasing the levels of soluble inhibitory factors and/or increase those of growth factors, with a greatest impact during the immediate postoperative period (32). Growth factors such as VEGF, as well as inflammatory mediators such as PGE₂, have been proposed as tumor growth promoting factors after tissue injury and during postoperative wound healing (33,34). These mediators could influence postoperative disease progression, depending on cancer type.

2.3. Surgery and dissemination of tumor cells

If surgery induces an accelerated growth of residual tumor cells, it may concern intra-operatively disseminated tumor cells in addition to previously dormant micro-metastases. Despite surgeons' efforts to anatomically isolate the primary tumor during resection, it has been shown that many patients present tumoural markers in the systemic circulation during

and following surgery. As an example, data from Pachmann show a surge in circulating epithelial cells after primary breast cancer surgery. Intriguingly, this surge occurs 3 to 7 days after surgery (35). A delayed increase of circulating like tumor cells (CTCs) after breast cancer surgery has also been reported by Daskalakis *et al.* Nevertheless, the origin of CTCs is not yet clarified, and even their surgery-induced increase in incidence is debated (36, 37). CTCs have been shown to be present in many cancer patients with apparent non-disseminated tumors (38). It implies that CTCs circulate in many cases, but with a low probability of finding a niche to survive, transmigrate out of the vessels, and grow as metastases (24). Moreover, CTCs may originate from the primary tumor, or from already disseminated cancer cells (in bone marrow or other tissues) (39). Surgery could promote recurrence either through an intra-operative dissemination or through the release of systemic factors that would influence previously disseminated tumor cells (8). Whatever their origin, CTCs have a prognostic value. Breast cancer patients with CTCs were shown to have earlier recurrences (40). In metastatic breast cancer, CTCs have been correlated with disease progression and mortality (41). Similar observations have been made in other cancers, such as colorectal carcinomas (34).

3. The complex role of inflammation

Whatever the moment of tumor cell dissemination, before, during, or after surgery, the mechanisms are similar. Before dissemination, some cytokines, particularly pro-inflammatory ones, play a central role in carcinogenesis, dedifferentiation and tumor growth (8,43). After the dissemination step, inflammation promotes proliferation of tumor cells by inhibiting apoptosis and increasing mitosis rate (8). However, inflammation also contributes to the initiation of antitumor immune responses, mainly through a cell-mediated immunity (CMI). Sensors of the innate immune system appear capable of detecting tumors at an early stage, for example through the recognition of soluble nucleic acids released from tumor cells. They trigger the local production of inflammatory cytokines that recruit immune cells such as lymphocytes, macrophages and dendritic cells. This Janus-like role of inflammation, i.e. positive and deleterious, is often referred to as the “paradox of inflammation” (42). While the concept of immune monitoring is now widely accepted, its role during the perioperative period is unknown (7) (Figure 2).

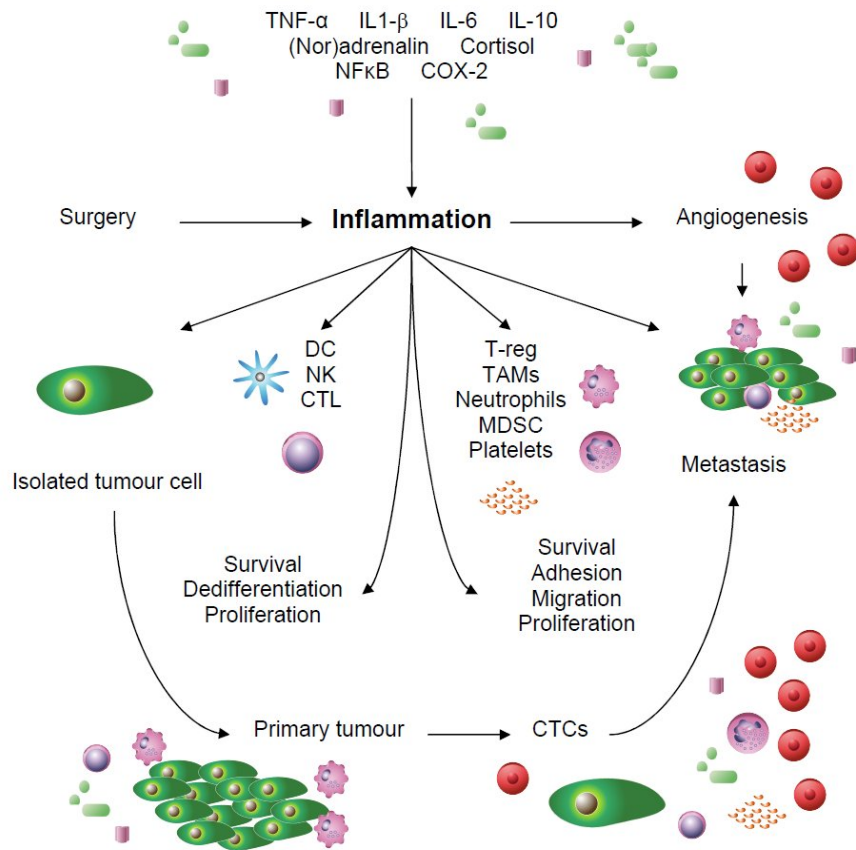


Figure 2. Surgery and inflammation are closely associated, and linked to mechanisms promoting tumour growth. At the time of the extirpation of the tumour, the incidence of circulating tumour cells (CTCs) depends of several mechanisms including inflammatory environment around the tumour itself. Inflammation promotes escape into the bloodstream, but also growth of metastases. Platelets can be involved in this dissemination process, by adhesion mechanisms and/or by the synthesis of mediators. Immune cells could both participate to the elimination of cancer cells (Natural Killer cells - NK, cytotoxic T lymphocytes - CTL, dendritic cells - DC) or to the suppression of the immune response (T regulator lymphocytes, T reg, tumour-associated macrophages and neutrophils, myeloid-derived suppressor cells - MDSC). COX-2 is overexpressed in tumour cells and in immune suppressor cells, like macrophages. Prostaglandins E2 (PGE2) could promote growth of the tumour directly and indirectly, via suppression of cellular-mediated immunity. The cytokines Interleukin-1 β (IL-1 β), Interleukin-6 (IL-6) and Tumor Necrosis Factor (TNF- α) can also suppress directly the activity of immune cells and promote the number and the activation of suppressor cells. Other factors, increased by surgery, accentuate this phenomenon, including (nor)adrenalin and cortisol levels. Anesthesia *per se* or by the impact against surgery-induced inflammatory process is able to interfere with many of these mechanisms.

3.1. Some inflammatory pathways promote tumor cell dissemination and proliferation

To disseminate and grow, tumor cells have to survive, proliferate and be fed by a blood supply after migration to peripheral tissues. Some inflammatory pathways promote all these mechanisms in both established tumors and disseminating CTCs. First, survival is facilitated by PGE2 secretion, and its direct action on G protein-coupled E-prostanoid receptors (EP), inducing the intracellular cAMP-dependent protein kinase A (PKA) pathway (43).

The PKA pathway can induce Bcl-2 expression (an oncogene inhibiting apoptosis), modulate nuclear factor-kappa (NFκB) secretion (indirectly via Toll-like Receptors - TLR) and activate peroxisome proliferator activated receptors (PPARs) (directly by phosphorylation and indirectly by stabilization of ligands-PPARs bindings) (8,43,44). In return, NFκB and TLR increase cell motility and migration by the induction of cytokines such as high mobility group B1 molecule (HMGB1). Second, a modified extracellular environment (reflected by the level of matrix metalloproteinase type 2 - MMP2), in the periphery and at a systemic level, is linked to facilitated migration of tumor cells. Finally, and at least in some cases, inflammatory pathways can promote proliferation, directly through the induction of phosphorylation of ERK associated to the liberation of HMGB1 (like in the case of necrosis), and indirectly via the promotion of angiogenesis (through VEGF and bFGF secretion and an IL-1beta-mediated NFκB-dependent expression of COX-2) (8,45,46,47). There is also a possible role for platelets, as well. Initially, platelets could promote the survival of CTCs, possibly through a protection from NK cells and resistance to shear stress. Aggregation of platelets around cancer cells inhibits in vitro NK cytotoxic activity and, at least with NK sensitive cells, tumour seeding in the target organs is reduced, in mice, after platelet depletion (48). After adhesion to endothelial cells and extravasation, platelet-derived factors such as platelet-derived lysophosphatidic acid (LPA) enhance metastasis, angiogenesis and tumor growth in an animal model of bone metastasis (49). Additionally, tumors produce a multitude of growth factors. For example, over-expression of COX-2 by colon or breast adenocarcinomas leads to PGE2 secretion and stimulation of epithelial cell proliferation, inhibition of apoptosis, stimulation of angiogenesis and production of mutagens (31,50,55).

3.2. Inflammation modulates immune defenses.

3.2.1. NK cells, cytotoxic T lymphocytes and dendritic cells

Natural Killer cells (NK), cytotoxic T lymphocytes (CTL) and dendritic cells (DCs) are crucial effectors against infection and are also involved in controlling tumor development (8,51). NK cells have caught attention of many investigators and have been extensively studied in animal models. Considered as major effector cells, but particularly vulnerable

during surgery, NK are already active at the early stages of tumor growth, before the activation of adaptive immunity by T cells (51,52). In animal models, NK activity is positively correlated with resistance to metastasis (28,29,30). Interestingly, these models have shown that the negative impact of surgery on NK activity can be prevented by analgesic techniques (53,54). In humans, a decrease of NK cytotoxicity during the perioperative period is well documented (52,55). However, there is no firm evidence of an effective role of NK activity yet, although a few reports suggest its role during the development of metastases (56). Cytolytic CD8⁺ T lymphocytes can specifically recognize and kill tumor cells. Quantitative and qualitative markers of tumor infiltration by T lymphocytes have been identified as strong prognostic factors, even if the causative link to outcome is still debated (57). PGE₂ inhibits cytokine production and cell survival. It can alter lymphocyte function through G protein-coupled E-prostanoid receptors and synthesis of cAMP (58). DCs play an important role in the development of anti-tumor T-cell responses. They present tumor-associated antigens to naive T-cells and stimulate their differentiation into cytolytic effectors. Their functions are inhibited by PGE₂, which can be produced by tumor cells or by monocytes and DCs to regulate the inflammatory cascade (59,63).

Noteworthy for the anesthesiologist, lymphocyte function is influenced by local or systemic inflammation, as well as by the central nervous system. This has been well described for NK cells (51,60). Concurrent with the inflammatory response to trauma (or surgery), the active pain regulatory mechanisms act through the midbrain periaqueductal gray matter, as well as through β -endorphin and adrenergic pathways. Norepinephrine inhibits NK activity through a direct effect on β -2 receptors, whereas the cholinergic system and the vagus nerve often counteract the sympathetic system. In turn, the brain influences prostaglandin secretion and the inflammatory cascade (60,61).

3.2.2. Tumor-associated immune cells

Immune cells play a major role in carcinogenesis (8). Tumor-associated neutrophils can “feed the tumor”. They produce reactive oxygen species (ROS), as well as bFGF, PGE₂ and VEGF (62). These factors lead to the development of more aggressive tumors, with accelerated growth. As described above, PGE₂ is produced either by immune cells, such as monocytes, macrophages and DCs, and by the tumor itself (59,63). COX-2, which is over-expressed in many tumor cells, and particularly in carcinomas, stimulates a direct PGE₂-induced increase of tumor growth and angiogenesis. In addition to a direct immunosuppressive effect on lymphocytes, tumor-associated macrophages (TAM) are attracted, as well as myeloid-derived suppressor cells (MDSC). TAMs have both pro- and anti-tumor effects, depending, at least

partially, on PGE2 secretion (46,47). In so far as COX-2 is over-expressed in many tumor cells, as well as in TAM, a potential vicious circle may be initiated. It involves TAM attraction, pro-tumor activity and PGE2 secretion. The granulocyte/neutrophil-like MDSC are a group of myeloid cells that accumulate in many cancer patients. They are morphologically similar to neutrophils, and their number and activity are rapidly and greatly increased by inflammation, notably by the cytokines IL1-beta and IL-6. These cells inhibit CTL and NK cell activities, blunting the antitumor immune responses (64) (Figure 2).

3.2.3. The paradox of inflammation and immune response

The paradox of inflammation, which induces both pro-tumor and anti-tumor mechanisms, has been clearly summarized by Dinarello (39). Cytokine production, production of ROS and PGE2, or the influx of macrophages and neutrophils are initially needed to induce immunity but can also contribute to its inhibition. Cytokines such IL1-beta and IL-8 contribute to an increase in the release of vascular adhesion molecules, production of VEGF and other growth factors, therefore promoting angiogenesis and metastasis. These same cytokines also recruit cells of the innate and adaptive immune system into the tumors.

The Society for Immunotherapy of Cancer, supported by the National Institute of Health (NIH), recently confirmed the importance of some inflammatory processes in cancer (8). The panel of experts recognized the importance of inflammatory and immune monitoring in cancer, and the need for specific interventions targeting some factors, like IL-1beta, TNF-alpha and PGE2. Increased levels of these mediators have been shown to be deleterious in terms of tumor progression (8). It is therefore possible that surgery-induced rise of these cytokines, of growth factors and of PGE2, at a time when CTCs are peaking in the bloodstream, has a long-term impact on patients overall survival.

For the anesthesiologist, the important message is that some inflammatory mediators produced by the tumor or by the host play a role in cancer and in relapses after surgery, and that there might be practical means to interfere with these mechanisms.

4. The place for perioperative interventions to lower cancer recurrence rate

4.1. Rationale sustaining pharmacological interventions

Since surgery has been suspected to accelerate tumor growth, anesthesiologists have tried to control the consequences of surgical trauma and avoid negative consequences of their

interventions (65). As a consequence, there is a rationale supporting the development of perioperative techniques to lower cancer recurrence rate associated with surgery.

First, opiates, and their avoidance with loco-regional analgesia, have been studied in this context, with discrepant results (10,11,12,13,14,15,16,66). Immunosuppressive effect of pain and opiates has been observed in animals and in humans. Cell-mediated immunity, and particularly NK activity, has been shown to be vulnerable to opiates. This effect is more pronounced when opiates are given at high doses. On the other hand, the largest immune suppression has been shown in operated animals where pain was not treated (53,54,55,56). In addition, morphinergic pathways have been suspected to directly increase tumor growth rate (67), possibly through an epidermal growth factor activation (68). Therefore, avoiding opiates could favorably impact cancer recurrence when an alternative pain management is possible and adequate. Loco-regional analgesia is one possibility, which could have a direct effect on the presumed deleterious components of the inflammatory response (69). Moreover, there is a large core of animal studies arguing for a positive influence of loco-regional analgesia on anticancer immunity (mostly NK activity, again) and metastasis occurrence (53,54,55,56). These results are in accordance with the possible influence of loco-regional analgesia (e.g. epidural) on several cytokines expressed during the perioperative period (70). Some of these cytokines could have a direct and/or indirect effect on the rapid progression of the disease after cancer surgery. Possible mechanisms include an influence on immune cells (both limiting and promoting tumor growth), tumor cell metabolism and capacity to increase mitosis rate, extracellular matrix and capacity to disseminate and migrate and, finally, angiogenesis mechanisms (Figure 1). All these mechanisms, favored by surgery, are suspected to be responsible for the magnitude of the first peak of relapse. Unfortunately, there are no specific studies focusing on the influence of opiates (or their avoidance) with loco-regional techniques on the first peak of relapse compared with the second peak. Most of studies analyzed the disease-free survival and/or the overall survival without highlighting a specific period. Moreover, several discrepancies, possibly methodology-related, appear in outcome studies. Briefly, there are problems with endpoint choices (e.g. definition of disease-free survival), over-fitting in multivariate analyses absence of an assessment of the peri-operative inflammatory response, molecular characteristics of the tumours and possible differences in the surgical techniques (e.g. due to different surgeons) or adjuvant therapies (e.g. endocrine treatment) playing also a role in early breast recurrences (16,70,71,72). Apart from opiates and loco-regional analgesia, another pharmacological intervention could be the use of non-steroidal anti-inflammatory drugs (NSAIDs). While largely under-investigated,

there is a strong argument for a possible role of NSAIDs in the prevention of cancer relapse after surgery.

4.2. *Non-steroidal anti-inflammatory drugs (NSAIDs)*

In addition to their opiate-sparing effect, NSAIDs present a promising anticancer profile with respect to the pathophysiological steps of tumor proliferation and dissemination. Most, but not all, of these effects may be mediated by the inhibition of PGE2 synthesis, described above as a major mediator in cancer. The inhibition of PGE2 synthesis could occur directly in tumor cells, impairing their capacity to survive, dedifferentiate and proliferate (59). This inhibition could increase the cytotoxic activity of NK and T lymphocytes, which is normally inhibited by PGE2. It could also decrease the influx and activity of tumor-associated immunocytes (58,63). Prior to the dissemination steps, NSAIDs could protect the integrity of the extracellular matrix, thereby reducing the liberation of tumor cells in the blood stream. During the dissemination process, NSAIDs may directly interfere with the survival capacity of CTCs, and their capacity to adhere to endothelial cells. This could also occur indirectly through the inhibition of platelet function, which is involved in all the following steps, including the angiogenesis processes. Finally, the growth of metastases disseminated before surgery or not, may be reduced through the same pathways, including inflammatory-related angiogenesis, immunocytes- and platelet-related release of growth factors, and survival and proliferation of tumor cells. All these mechanisms were described above and summarized in Figure 2 (42,46,47,49,58,59,63). These therapeutic effects of NSAIDs in cancer have been described for various carcinomas, with a great concordance between studies. While the role of NSAIDs in cancer prevention remains unclear, this is not related to a lack of data on their anticancer effect but, rather, to side effects associated with chronic administration (73, 74).

In June 2010, we reported data from a retrospective study of 327 consecutive breast cancer patients treated by one surgeon in one Belgian hospital, comparing various perioperative analgesic and anesthetic regimens (3). Patients were treated by a mastectomy and conventional adjuvant therapy, and did or did not receive the NSAID ketorolac intra-operatively. The average follow-up was 27.3 months with range of 13-44 months. Intra-operative ketorolac administration was associated with a significantly higher disease-free survival during the first 5 years after surgery. With Retsky and our collaborative group, we have recently reanalyzed this database, focusing on the differential impact of ketorolac on early and late recurrence peaks (75). Using hazard smoothed plots analysis, we confirmed that the expected prominent early relapse peak is all but absent in the ketorolac group. The few events in the ketorolac group show a small bump during the first 10 months, and then a slow

rise until the fourth year. We observed an approximately six fold decrease of the early peak hazard. If this observation holds up in a larger and randomized trial, it would mean that using this safe and effective anti-inflammatory agent at the time of surgery could prevent most early relapses.

Similar retrospective analyses could be conducted on other cohorts, focusing on the impact of perioperative interventions in oncological surgery. Noteworthy, in a series of patients, we compared loco-regional anesthesia *and* systemic opiates. We observed that, if present, the impact of a perioperative technique affects primarily the early recurrence peak (9,10,11,12,13,14). We cannot firmly conclude to a causal relationship, but a better understanding of the pathophysiology of cancer during the perioperative period is urgently needed.

Conclusions and future

The anesthesiologist takes care of cancer patients at a critical time of their life. In addition to local and systemic disorders caused by the neoplastic disease, surgery is an additional source of disequilibrium for factors influencing tumor growth. There is a need for a more profound understanding of tumor biology during the perioperative period, and for an analysis of the impact of various anesthetic maneuvers on tumor recurrences. Assessments of the evolution of perioperative inflammation and immunity could identify several groups requiring distinct anesthetic managements, in order to minimize their risk of relapse. The anesthesiologist's role in the oncology 'medical team' could substantially increase.

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SECTION 1

Do intraoperative analgesics influence breast cancer recurrence after mastectomy? A retrospective analysis.

This study is the first retrospective analysis we conducted. This was of prime importance to explore the possible influence of analgesic techniques on cancer outcome.

This study shows the effect of the non-steroidal anti-inflammatory drug ketorolac on clinical outcome after breast cancer surgery, as suggested from many years in animals.

Do Intraoperative Analgesics Influence Breast Cancer Recurrence After Mastectomy? A Retrospective Analysis

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ABSTRACT

BACKGROUND: Whether intraoperative analgesics have an impact on postoperative cancer recurrence is unknown. Some investigations suggest that the opioids could favor relapse and that regional analgesia and nonsteroidal antiinflammatory drugs could improve cancer prognosis. We retrospectively reviewed our series of breast cancer surgery patients.

METHODS: This retrospective study included 327 consecutive women who underwent mastectomy with axillary dissection for breast cancer. The main objective was to compare the incidence of cancer recurrence among patients who received different analgesics during surgery.

RESULTS: Perioperative characteristics, cancer prognostic factors, and the length of surgery were comparable regardless of the analgesics administered. Univariate and multivariate analyses showed a lower cancer recurrence rate when ketorolac was given before surgery ($P=0.019$). Other analgesics (sufentanil, ketamine, and clonidine) were not associated with a significant reduction in cancer recurrence rates in our series.

CONCLUSION: This retrospective analysis suggests that intraoperative administration of ketorolac decreases the risk of breast cancer relapse compared with other analgesics.

Surgery remains the keystone in the treatment of solid neoplastic tumors including breast cancer. Paradoxically, the perioperative period represents a high risk of metastases development.¹ Several factors may account for this phenomenon, including profound depression of antitumoral cellular immunity. Depression of this cellular immunity in the postoperative period is at least partially linked to the metabolic and hormonal changes induced by the “stress reaction” to surgery.² Analgesics may influence immunity and tumor development either directly by interfering with cellular mechanisms (e.g., cell apoptosis) or indirectly by interacting with the endocrine and sympathetic systems.

In animal models, morphine-based analgesia reduces the number of pulmonary metastases after surgery.³ However, in humans, recent retrospective data suggest that opioid-based perioperative analgesia is associated with reduced recurrence-free survival, when compared with regional techniques for breast and prostate cancer surgery.^{4,5} Very few studies have evaluated the possible immune effect of the other analgesics or antihyperalgesics used for “balanced” perioperative analgesia such as the nonsteroidal antiinflammatory drugs (NSAIDs), ketamine, and clonidine.

Currently, whether perioperative analgesia has an impact on postoperative cancer recurrence remains largely unknown.¹ Therefore, we performed a retrospective analysis of medical records to compare the incidence of local recurrence and metastatic disease after mastectomy in patients with breast cancer who received different intraoperative analgesics (i.e., sufentanil, ketamine, ketorolac, and clonidine).

METHODS

Patients and Procedures

After approval from the Ethical Committee of the St-Luc Hospital, Brussels, Belgium, we reviewed the medical records of 327 consecutive patients who underwent mastectomy with axillary dissection between February 2003 and September 2008. The size of this convenience sample was mainly due to logistical reasons (availability of the medical records), but it also maintained the homogeneity in the oncological treatments among the patients. Patients with previous ipsilateral surgery for breast cancer were excluded.

Indications for mastectomy with axillary dissection were defined according to international recommendations and guidelines.^{6,7} These indications were discussed every week by the multidisciplinary board of our breast clinic and regularly updated and adjusted with new international recommendations and literature data. All mastectomies were performed by the same surgeon (MB) and jointly followed by the surgeon and the same oncologist (J-PM). Chemotherapy, radiotherapy, and endocrine therapy were performed according to international expert consensus (ninth and 10th St-Gallen consensus).^{8–11}

During the first 2 postoperative years, medical consultation occurred every 3 months, then every 6 months for 3 additional years, and then once a year. For all operations, general anesthesia was induced with sufentanil ($0 - 0.2 \mu\text{g}/\text{kg}^{-1}$) and a hypnotic, sodium thiopental ($4 \text{ mg}/\text{kg}^{-1}$) or propofol ($2-3 \text{ mg}/\text{kg}^{-1}$). After insertion of a laryngeal mask airway, anesthesia was maintained with continuous infusion of propofol, plus sevoflurane or desflurane in an oxygen/air mixture. The type, dosage, and combination of intraoperative analgesic therapy were left to the discretion of the 2 anesthesiologists in charge. Total doses administered were as follows: sufentanil ($0-0.5 \mu\text{g}/\text{kg}^{-1}$); preincisional clonidine ($0-6 \mu\text{g}/\text{kg}^{-1}$); and preincisional ketamine ($0-0.5 \text{ mg}/\text{kg}^{-1}$). Ketorolac, when administered before the incision, was used in IV doses of 20 mg in patients $<60 \text{ kg}$ body weight and 30 mg in patients $>60 \text{ kg}$.

In the postanesthesia care unit, postoperative analgesia consisted of IV piritramide titrated until the visual analog scale scores were lower than 4 on a scale anchored with 0 as “no pain” and 10 as “the worst pain ever experienced.” During the first 48 postoperative hours, all

patients received acetaminophen, 3 to 4 g/d. Oral diclofenac 50 mg was administered 3 times a day for 3 days as necessary, in the absence of contraindication (gastric, renal, or advanced age). No additional opioid was administered.

Primary End Point

The main objective of this study was to determine the effect, if any, of the administration of different intraoperative analgesics (sufentanil, ketamine, clonidine, and ketorolac) on cancer recurrence. Consequently, our primary end point for this analysis was the length of recurrence-free survival through February 2009. Recurrence-free survival was measured from the date of surgery to the date of first recurrence, death due to oncological cause, or to the date of last follow-up, whichever occurred first. Recurrence was defined as clinical evidence of local recurrence or development of metastases confirmed by radiological examination(s). Patients lost to follow-up or those remaining disease free at the time of analysis were censored in the statistical model at the date of the last follow-up (administrative censoring). Patients who died of nononcological causes were censored at the date of death (assuming noninformative censoring).

Data Collection

The following data were obtained from the medical records: perioperative (demographic) characteristics, tumor size, histological tumor grade, histopathological type, estrogen and progesterone receptor status, epidermal growth factor receptor type 2 (HER-2) expression, extent of axillary node disease, and administration of perioperative or post-operative adjuvant chemotherapy, radiotherapy, or endocrine therapy. The patient's current status was determined by the most recent follow-up in our breast cancer clinic through February 2009. Lost to follow-up was defined as patients who lacked follow-up for 3 months after the last clinic visit attended date. The Nottingham Prognostic Index was calculated based on the histological findings: 0.2 (tumor size), histological grade (1: grade 1, least aggressive tumor appearance; 2: grade 2, intermediate appearance; and 3: grade 3, most aggressive appearance), axillary lymph node involvement (1: no axillary lymph nodes involved, 2: up to 3 axillary lymph nodes involved, and 3: >3 axillary lymph nodes involved).⁴ The type and total dose of the analgesics administered intraoperatively were obtained by reviewing the electronic intraoperative and postoperative records. The duration of the surgical procedure, and type and doses of hypnotic administered were also noted.

Statistical Analysis

Patient baseline characteristics are presented as mean $\pm$ SD, median (interquartile 25–75), or numbers (percentage). Univariate Cox model and log-rank test were used to assess the

potential effect of these baseline characteristics and to investigate an eventual effect of the administration of the analgesics on recurrence-free survival probability. Kaplan-Meier analyses were used to estimate recurrence-free survival probabilities. Variables considered for the univariate analyses were tumor size, histological grade and lymph node involvement,¹² age, height, weight, length of surgery, Nottingham Prognostic Index, hormonal receptor status (estrogen and/or progesterone positive), HER-2 expression, hypnotics (propofol, sevoflurane, or desflurane), and postoperative analgesics (synthetic opioid piritramide and diclofenac).

After univariate analyses, the Cox regression model was used for multivariate analysis while adjusting for any baseline factors and intraoperative or oncological factors related to the outcome in the univariate analyses ($P < 0.05$). A single model was conducted including all the analgesics simultaneously to assess the effect of each analgesic while adjusting for taking one and not another analgesic. We then used stepwise manual backward regression, and all significant factors at a value of $P > 0.05$ were retained in the final model. STATISTICA (data analysis software system, 2004) version 7 (StatSoft, Tulsa, OK) was used for all analyses.

RESULTS

Patients and Procedure Characteristics: Histopathological Findings

The data from 327 consecutive patients who underwent mastectomy with axillary dissection were reviewed. Eight patients were excluded because of intrathoracic tumor expansion, incomplete surgical resection, and/or perioperative metastases. The data from the 319 remaining patients were analyzed. Patients, histological findings, and duration of the surgery are summarized in Table 1. Postoperative complications included wound infection in 13 patients (4%) and significant hemorrhage requiring transfusion and re-operation in 1 patient (0.3%).

The median follow-up time was 27.3 months (13–44 months). Recurrence was noted in 35 patients (11%), and 17 patients (5%) died of oncological causes during the follow-up period. Deaths from nononcological causes were rare (3 patients, 0.9%), distributed equally according to the type of treatment, and not related to treatment (2 due to pneumonia and 1 due to cardiovascular disease). Sixteen patients (5%) were lost to follow-up.

Intraoperative and Postoperative Analgesics

The intraoperative analgesics included sufentanil, ketorolac, clonidine, and ketamine. Table 2 summarizes the proportion of patients receiving the various drugs. Clonidine and ketamine were often combined for their opioid-sparing effects and were associated with smaller doses of sufentanil. Half of the patients who received sufentanil also received ketorolac. All

postoperative analgesics (except acetaminophen) are also listed in Table 2.

Table 1. Patient Characteristics

Patients and procedure characteristics: histologic findings (N = 319)	
Age (y)	59 ± 14
Weight (kg)	68 ± 15
Height (cm)	163 ± 9
Tumor size (mm)	34 ± 24
Histologic grade	
1	30 (9%)
2	111 (35%)
3	178 (56%)
Lymph node invasion	
1 (none)	151 (47%)
2 (1–3 positive lymph nodes)	99 (31%)
3 (>3 positive lymph nodes)	69 (22%)
Nottingham Prognostic Index	11 ± 5
Hormonal receptor status	
Estrogen positive	262 (82%)
Progesterone positive	280 (88%)
HER-2 expression	172 (54%)
Duration of surgery (min)	90 ± 26

Data are presented as mean ± SD or as numbers (percentage).

Table 2. Number of Patients Receiving Intra- and Postoperative Analgesics and the Doses Administered

	Number of patients	Doses
Ketorolac	175 (55)	20 mg [0–25]
Sufentanil	227 (71)	15 µg [0–18]
Clonidine	184 (58)	150 µg [0–185]
Ketamine	166 (52)	10 mg [0–20]
Piritramide	159 (50)	2 mg [0–4]
Diclofenac	223 (70)	50 mg [0–50]

Intravenous ketorolac, sufentanil, clonidine, and ketamine were administered intraoperatively. Intravenous piritramide was administered in the postanesthesia care unit until visual analog scale score <4. Diclofenac was administered if necessary during the first 48 postoperative hours. Data are presented as numbers (percentage) and median [interquartile 25–75].

Association Between Use of Analgesics and Cancer Recurrence: Univariate and Multivariate Analyses

Potential confounders, including perioperative patient characteristics, cancer prognostic factors, and length of surgery, were equally distributed over the groups of patients treated with the different analgesics (data not shown). One exception is the younger age of the patients receiving ketorolac (56 +/- 12 vs 63 +/- 14 years) ($P=0.02$).

In univariate analysis, intraoperative administration of ketorolac was associated with longer recurrence-free survival ($P=0.019$). Cancer recurrence was more frequent in patients who did not receive intraoperative ketorolac (17%, $n=25$ of 145 vs 6%, $n=10$ of 174; $P=0.001$). No significant change in the rate of cancer recurrence was found in the patients who received sufentanil, clonidine, ketamine, and other drugs ($P>0.05$) (Table 3, Fig. 1). Univariate analysis also showed that age ($P=0.004$), histological grade ($P=0.006$), lymph node invasion ($P<0.001$), and Nottingham Prognostic Index ($P=0.048$) were significantly associated with recurrence-free survival (Table 3).

Table 3. Univariate Analyses of Demographic Characteristics, Cancer Prognostic Factors, Length of Surgery, and Intraoperative Analgesics for Breast Cancer Surgery

	Beta	Hazard ratio	95% CI	P
Age	0.04	1.04	1.02–1.42	0.004
Height	0.02	1.02	1–1.03	0.054
Weight	0.26	1.03	0.98–1.07	0.31
Tumor size	0.006	1	1–1.1	0.36
Histologic grade	0.96	2.62	1.92–3.32	0.006
Lymph node invasion	0.95	2.6	2.16–3.04	<0.001
Nottingham Prognostic Index	0.05	1.05	1–1.1	0.048
Hormonal receptor status				
Estrogen positive	−0.81	0.44	0–1.15	0.25
Progesterone positive	−0.36	0.69	0–1.39	0.45
HER-2 expression	−0.02	0.98	0.33–1.63	0.94
Duration of surgery	−0.001	0.99	1–1.01	0.94
Hypnotics				
Thiopental	0.002	1	1–1.1	0.19
Propofol	0.15	1.17	0.87–1.47	0.59
Desflurane	0.36	1.44	0.69–2.2	0.36
Sevoflurane	−0.54	0.58	0–1.33	0.16
Ketorolac	−0.89	0.41	0–1.19	0.019
Sufentanil	−0.31	0.73	0–1.83	0.57
Clonidine	0.77	2.17	0.33–3.01	0.63
Ketamine	−.056	0.57	0–1.49	0.23
Postoperative analgesics				
Piritramide	−0.49	0.61	0–1.31	0.16
Diclofenac	−0.03	0.97	0.85–1.19	0.57

Data are presented as factor effect (beta) estimated from univariate Cox regression model, hazard ratio (HR) and associated 95% confidence intervals (CIs), and log-rank *P* value.

The Cox regression model was used for multivariate analysis while adjusting for these factors. Using stepwise manual backward selection, age, histological grade, and lymph node invasion were retained as risk factors in our multivariate analysis model (Table 4).

When adjusting for age ($P=0.02$), histological grade ($P=0.015$), and lymph node involvement ($P<0.001$), the risk of cancer recurrence remains significantly lower after the intraoperative administration of ketorolac ($P=0.019$). No significant effect was found with sufentanil, clonidine, and ketamine ($P>0.05$) (Table 4). A single model was constructed to test all the analgesics simultaneously, to assess the effect of each analgesic while adjusting for the others, and to consider the risk of potential confounder effects in the case of drug

combinations.

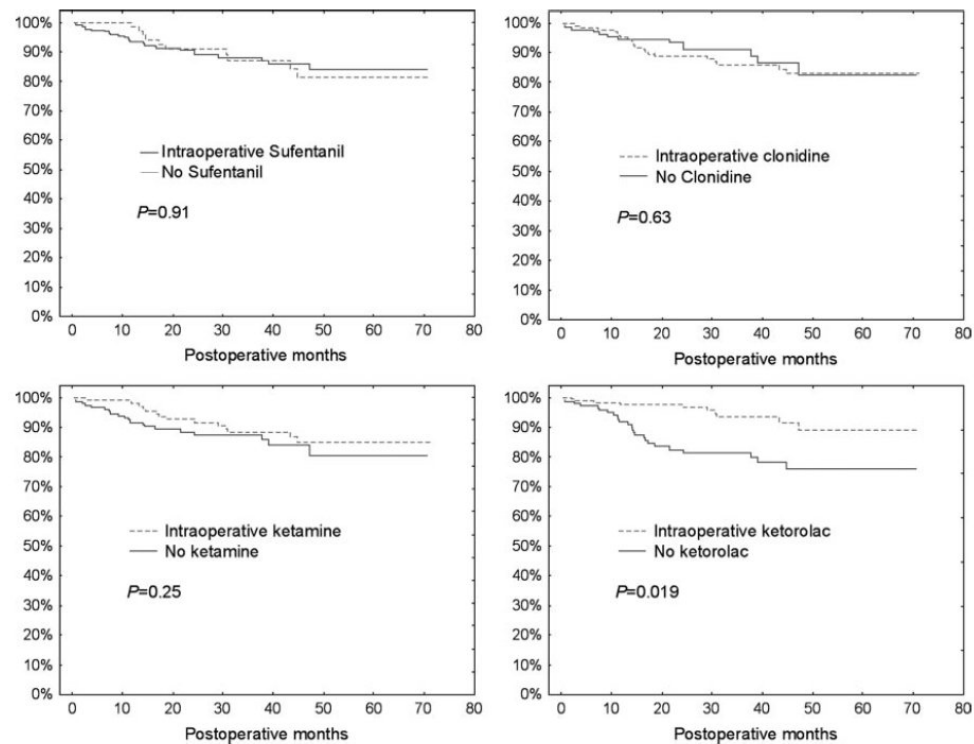


Figure 1. Kaplan-Meier recurrence-free survival estimated for 319 patients receiving (or not receiving) intraoperative analgesics (sufentanil, clonidine, ketorolac, and ketamine). Univariate analysis by log-rank tests.

Table 4. Multivariate Association with Cancer Recurrence After Mastectomy: Cox Regression Model

	Beta	Hazard ratio	95% CI	P
Age	0.03	1.03	1–1.06	0.02
Histologic grade	0.85	2.34	1.67–3.01	0.015
Lymph node invasion	0.83	2.28	1.87–2.69	<0.001
Ketorolac	−0.98	0.37	0–0.79	0.019
Sufentanil	−0.31	0.73	0–1.83	0.57
Clonidine	0.77	2.15	0.74–3.56	0.08
Ketamine	−0.56	0.57	0–1.49	0.23

Data are presented as factor effect (beta) estimated from multivariate Cox regression model, hazard ratio (HR) and associated 95% confidence interval (CI), and P value.

DISCUSSION

This retrospective analysis suggests an association between a reduced risk of breast cancer recurrence after surgery and the perioperative administration of ketorolac. This reduced risk was not observed when other intraoperative analgesics were administered. In contrast with previous data suggesting a negative influence of opioids on cancer-related immunity,¹³ sufentanil had no deleterious effect on cancer recurrence. One reason may be the doses used in our patients, which are relatively small in comparison with those in other series.¹⁴ This is consistent with the fact that opioid-induced immunosuppression is dose dependent.¹³

The alpha-2-agonist clonidine enhances hypnotics, optimizes postoperative analgesia, and stabilizes intraoperative hemodynamic variables. This drug potentially interferes with immunity via adrenergic antiinflammatory pathways and by a central analgesic effect that reduces sympathetic tone¹⁵; these effects are immunosuppressive in the perioperative period.² The doses administered were chosen based on the patients' physical conditions and the fact that mastectomy is classically considered a minor surgery¹⁴ that induces a relatively limited "stress reaction."¹

Ketamine is a drug widely used in the perioperative period. At subanesthetic doses, it prevents hyperalgesia and enhances analgesia.¹⁶ Ketamine, similar to the other analgesics, interferes with natural killer (NK) cell activity. It possesses antiinflammatory properties in humans during the postoperative period¹⁷ or in severe sepsis.¹⁸ In our series, ketamine was not associated with improved onco-logical outcome.

When considering the administration of ketorolac, these results suggest that, given just before the surgery, it was associated with a lower risk of breast cancer relapse. By multivariate analysis, age was identified as a potential confounder. Indeed, advanced age was considered a relative contraindication for NSAIDs and was consequently associated with a lower use of NSAIDs. After adjustment for the significant oncological predictors (histological grade and lymph node invasion) and the potential confounder (age), the association between ketorolac administration and the lower risk of cancer recurrence remained significant. Our observation is consistent with the results obtained in previous studies that investigated the influence of NSAIDs on antitumoral immunity. These studies identified the role of prostaglandins in immunity and inflammation,^{19,20} and the positive effect of NSAIDs on immunity and against cancer progression in both animals and humans.²¹⁻²⁶ Indeed, cyclooxygenase-2 (COX-2) inhibitors are active in some models of breast cancer, and COX-2 could play a role in tumor development.²⁴ In fact, overexpression of COX-2 in breast cancer leads to stimulation of epithelial cell proliferation, inhibition of apoptosis, stimulation of

angio-genesis, immune suppression, and increases the production of mutagens.²³ This favors breast tumor growth and increases the risk of cancer relapse. These effects are mostly mediated by prostaglandins and specifically prostaglandin E₂ (PGE₂).^{19,24} PGE₂ is released by monocytes and dendritic cells to regulate the inflammatory cascade and profoundly depresses cellular antitumoral immunity, i.e., NK cell activity.²⁴ The PGE₂-induced suppression of NK cell activity is carried via membrane receptors that trigger the synthesis of cyclic adenosine monophosphate, which interferes with the cytotoxicity of NK cells. This suppression of NK cell activity dissipates quickly after removal of prostaglandins. The time course of postoperative immunosuppression extends from the first postoperative hours to a few days. As a consequence, the optimal window for administration of a drug that acts on NK cells may be very short, ideally just before surgery. This time-specific effect may explain the effect of intraoperative ketorolac and the absence of effect of postoperative diclofenac in our retrospective study.

Another explanation is a specific effect of ketorolac. Indeed, inhibition of COX-2 is necessary to counteract the deleterious effects of PGE₂,²⁵ but, although all NSAIDs act against the growth of tumors, they are probably not equivalent in their antitumor effects. Alternative targets, such as the tumor-associated reduced nicotinamide adenine dinucleotide oxydase (tNOX), are possibly involved in this anticancer effect. The existence of tNOX explains the fact that some cancer cell lines lacking COX-2 respond to certain NSAIDs but not to others, suggestive of additional COX-2-independent antitumor activities.²⁶ Further studies are required to explore the differences between the NSAIDs and their antitumor effects in this context.

When analyzing the timing of cancer recurrence, the greatest difference between patients receiving ketorolac and those not receiving it appears between 9 and 18 months. This early cancer recurrence confirms our observation that intraoperative administration of ketorolac improves the control of residual neoplastic disease. Cancer relapse is a consequence of intraoperative spread of tumor cells in the bloodstream, local recurrence, or previously disseminated micrometastatic dormant cells.²⁷ Inducing or maintaining dormancy in these neoplastic cells is a major goal for the adjuvant therapeutics, e.g., endocrine therapy. The control of residual disease limits the risk of early recurrence. It explains why recurrences occur gradually during the first 10 to 15 years in women treated with endocrine therapy, whereas in women not treated with endocrine therapy (i.e., with estrogen receptor-negative tumors), the majority of cancer relapses occur in the first 2 years.²⁷

Our study is potentially limited by the retrospective design and the exploratory nature of

our analyses. Uncontrolled and unrecognized biases are frequent in retrospective studies. All known variables that influence cancer outcome were analyzed, but this does not exclude unrecognized bias. Overfitting, i.e., having poor predictive performance or exaggerating minor fluctuations in the data, is also a well-known problem of prognostic factor analyses, and validation against an external database is always advisable. The collection of data was systematic and the investigators (PF, JV, BN, and MDK) used the same methodology. The quality of the follow-up (MB and J-PM) and the accessibility of data permitted us to ascertain all variables. Nevertheless, to confirm these data, prospective well-conducted and multicenter studies are required. Although never reported as immunosuppressive, we did not investigate the effect of acetaminophen, because it was prescribed systematically during the postoperative period.

In conclusion, our data support a positive effect of ketorolac administered preoperatively for breast cancer surgery. These data are consistent with previous animal and human preclinical studies that suggested an anticancer effect of preoperatively administered NSAIDs.

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SECTION 2

Do intraoperative analgesics influence oncological outcome after radical prostatectomy for prostate cancer?

This study is the second retrospective analysis we did.

In this study concerning prostate cancer patients, a completely different population than breast cancer patients, we aimed to investigate the influence of anaesthetic/analgesic techniques on cancer outcome.

This study is an important observation leading to further discussion on the possible influence of host/tumour interactions (including the inflammatory response) on the potential anticancer effect of ketorolac.

Do intraoperative analgesics influence oncological outcomes after radical prostatectomy for prostate cancer?

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Conflict of Interest: The authors attest no conflict of interest.

ABSTRACT

Background: The potential impact of intraoperative analgesics on oncological outcome after radical prostatectomy is debated. Some investigators have suggested that use of opioids favour relapse, whereas regional analgesia and NSAIDs improve oncological outcomes.

Objective: To evaluate the impact of intraoperative analgesia (epidural and intravenous) on the incidence of biochemical recurrence-free (BRF) survival.

Design, setting and participants: This retrospective study includes 1111 consecutive retropubic radical prostatectomies (RRPs) for localised prostate cancer, performed between 1993 and 2006. Median follow-up was 38 months (interquartile range 16–69). BRF survival probabilities were compared with log-rank tests and the Cox regression model.

Main outcome measures and results: Epidural analgesia was used in 52% of patients, intravenous ketorolac in 25%, sufentanil in 97%, clonidine in 25% and ketamine in 16%. Univariate and multivariate analyses showed that intravenous sufentanil significantly reduced BRF survival rate, hazard ratio 7.78 [95%confidence interval (CI) 5.79, 9.78], for extracapsular extension stage pT 2 or less, hazard ratio 0.44 (95%CI 0.12, 0.75), Gleason score at least 7, hazard ratio 1.96 (95% CI 1.65, 2.26), positive margin, hazard ratio 1.87 (95%CI 1.58, 2.02) and lymph node involvement, hazard ratio 1.77 (95% CI 1.27, 2.27, $P>0.05$). In contrast, neither epidural analgesia nor other analgesics were associated with a statistically significant effect ($P>0.05$).

Conclusion: This retrospective analysis suggests that intraoperative sufentanil administration is associated with an increased risk of cancer relapse after RRP, whereas epidural analgesia, with local anaesthetic and opioid, was not associated with a significant effect.

INTRODUCTION

Prostate cancer (PCa) is the most common malignancy in men.¹ Retropubic radical prostatectomy (RRP) has been the treatment of choice for localised disease for the last 2 decades.² Despite improvements in the surgical technique, RRP is not curative in every patient, as overall 35% will experience a prostate-specific antigen (PSA) recurrence,³ a commonly accepted surrogate marker for metastatic progression and disease-specific survival.^{4,5}

The risk of cancer recurrence after RRP depends on extension stage, PSA, biopsy Gleason score and other tumour characteristics (positive margin, lymph node and/ or involvement of

seminal vesicles). Most of these are direct or indirect markers of invasion outside the prostate, pointing to a more advanced disorder.⁵

Over the years, there has been a debate as to whether the surgical procedure itself, an external factor, negatively influences the recurrence rate.⁶ It is known for instance that PCa cells become blood borne as a result of surgery, but the impact on recurrence rate has never been demonstrated. Several other hypothetical factors also fuel this theory, including the fact that surgery is associated with a profound depression of anti-tumoural cellular immunity.⁶ This is of paramount importance, as the analgesics used during anaesthesia have been shown to influence immunity and tumour development, either directly by interfering with cellular mechanisms (e.g. cell apoptosis) or indirectly by interaction with the endocrine and sympathetic systems.⁷ Pain itself is known to suppress immunity in several situations, including cancer, and it is of interest that different analgesic techniques may differentially influence cancer prognosis, at least in animal models.⁸

Recent retrospective surveys conducted in patients undergoing surgical extirpation of breast, prostate or colorectal cancer have suggested that opioid-based perioperative analgesia was associated with shorter recurrence-free survival than regional techniques. These studies had limited cohorts of patients, and did not report the effect of other non-opioid analgesics, that might also interfere with immune mechanisms.⁹⁻¹⁴ For example, it has been suggested that the NSAID, ketorolac, has a positive impact on recurrence-free survival after breast cancer surgery.¹⁵

To increase our understanding of the role of intra-operative analgesics on postoperative cancer recurrence, we investigated, in a large series, the possible impact of all the components of intraoperative analgesia on biochemical recurrence-free (BRF) survival after RRP.

METHODS

Ethics

Ethical approval for this study (Ethical Committee N/REF 2010/15MAR/085, national registration number B40320108384) was provided by the Ethical Committee CEBH of the Universite' Catholique de Louvain (Chairperson Prof. Dr J.M. Maloteaux) on 29 March 2010.

Patients and procedures

We retrospectively reviewed the charts of 1111 consecutive patients who, between January 1993 and August 2006, underwent RRP for localised prostatic cancer defined as a clinical T1-2 (tumour extension stage), N0 (no lymph node involvement), M0 (no metastasis), based on the preoperative digital rectal examination (DRE), abdomino-pelvic computed tomography

and Tc-99m bone scan that were performed in all patients.

All radical prostatectomy specimens were uniformly processed and evaluated by a single experienced uro-pathologist. Pathological stage was defined using the TNM 2002 classification (based on the Tumour extension stage, lymph Node involvement and presence of Metastasis, using the detailed pathological report). Gleason score was calculated from the same detailed report, including description of the Gleason grade of the individual tumour foci. Gleason grades were retro-spectively adapted to comply with the 2005 International Society of Urological Pathology (ISUP) Consensus.¹⁶ Patient follow-up consisted of PSA tests and DRE, tri-monthly during the first 2 years and for every 6 months thereafter with the interval extending over time. Biochemical recurrence (BCR) is defined as a sequence of rising PSA values at least 3 months apart with a minimum value of 0.2 ng ml⁻¹. BRF is defined as the interval between the date of surgery and the date the PSA was recorded at 0.2 ng ml⁻¹ or above.⁴

All procedures were performed under general anaesthesia. An epidural catheter was placed, before the induction of general anaesthesia, at a low thoracic level (typically the T9-T10 level) under local anaesthesia, depending on the preference of the anaesthesiologist and the presence of a contraindication or the patient's refusal. Anaesthesia was induced with thiopental or propofol, with or without sufentanil, and maintained with a continuous infusion of propofol, isoflurane, sevoflurane or desflurane in an oxygen-air mixture.

In the epidural space, local anaesthetics were infused (lidocaine, bupivacaine) in combination with an opioid, sufentanil, 5–10 mg, with or without clonidine, 10–300 mg. A preoperative bolus dose was followed by a continuous intraoperative and postoperative infusion for 48–72h.

Primary endpoint

The main objective of this study was to determine the benefit, if any, of the administration of epidural analgesia

and/or intravenous intraoperative analgesics (sufentanil, ketamine, clonidine and ketorolac) on BRF survival in univariate and multivariate analyses. Patients who died from non-oncological causes were censored at the date of the death (assuming non-informative censoring).

Data collection

To avoid inclusion biases and to ascertain the highest methodological quality, we included only patients with surgery after the beginning of 1993 when epidural analgesia became standard for RRP, and sufentanil replaced fentanyl. The following data were obtained from

the medical records: baseline preoperative characteristics; tumour size; Gleason score (adapted to TNM 2002 classification); margin; preoperative PSA; transfusion; and administration of preoperative or postoperative adjuvant chemotherapy, radiotherapy or endocrine therapy. The patient's current status was determined by the most recent follow-up in our cancer clinic before the end of August 2006.

Statistical analysis

Patients' characteristics are presented as mean $\pm$ SD, median (interquartile range, IQR) or number (percentage) as appropriate. Recurrence-free survival probability curves were estimated using Kaplan–Meier analyses. A univariate Cox regression model and log-rank test were used to assess the potential impact of these baseline characteristics and to investigate an eventual effect of the administration of the analgesics on recurrence-free survival probability. Variables considered for the uni-variate analyses were as follows: use of epidural analgesia; age; tumour size; Gleason score; margin; preoperative PSA; transfusion; period of surgery (before 1 January 2000 or after); hypnotics (propofol or sevoflurane or desflurane); and intravenous analgesics.

After univariate analyses, the Cox regression model was used for multivariate analysis while adjusting for any baseline factors and intraoperative or oncological factors. We included in the model all baseline characteristics and analgesics and then used a stepwise manual backward selection procedure. Each different type of analgesic and all other factors significant at P value less than 0.05 were retained in the final model.

STATISTICA (data analysis software system) version 7 (Statsoft Inc. 2004, Tulsa, USA) was used for all the analyses.

RESULTS

Patients' characteristics, procedures and histopathological findings

Data from 1111 consecutive patients were reviewed. Median follow-up time was 38 (IQR 16–69) months. Patients' characteristics and histological findings are summarised in Table 1. Neo-adjuvant had been administered to 78 (7%) and adjuvant androgen deprivation therapy to 11 (1%). Adjuvant radiotherapy had been administered to 156 (14%) patients, 132 being treated in the framework of the EORTC22911 trial.¹⁷ Another 50 patients had received adjuvant radiation therapy outside the protocol. BCR occurred in 189 (17%), and 10 (1%) patients died from documented PCa progression during the follow-up period. Nine patients died from oncological causes linked to other cancers (1%). Fifty-seven other patients died: 12 from non-oncological causes; nine from cardiovascular disorders; two from sepsis; one from

neurodegenerative diseases; and 45 from unknown reasons, but without biological PSA recurrence. Type, frequency and mean doses of intraoperative analgesics used and the number of patients benefiting from epidural analgesia are shown in Table 2.

Table 1 Patients characteristics

Patients and procedure characteristics, histological findings		N = 1111
Age (years)		65 ± 7
All cause mortality		67 (6%)
Recurrence		189 (17%)
Gleason score		
0–6		844 (76%)
≥7		267 (24%)
Lymph node involvement		43 (4%)
Extracapsular extension stage		
pT≤2		722 (65%)
pT≥3		389 (35%)
Seminal vesicles involvement		48 (5%)
Preoperative PSA		
0–10		695 (63%)
10.1–20		322 (29%)
>20		89 (8%)
Unknown		5 (0.5%)
Positive margin		329 (30%)
In pT≤2		152 (21%)
In pT≥3		183 (47%)

Data are presented as mean ± SD or as number (percentage). PSA, prostate-specific antigen.

Table 2 Number of patients receiving intraoperative epidural analgesia, intravenous analgesics, hypnotics and the doses administered

	Number of patients	Doses
Thiopental	36 (8%)	379mg (±127)
Propofol infusion/ halogenated vapours	378 (34%)/733 (66%)	
Ketorolac	278 (25%)	24mg (±7)
Sufentanil	1078 (97%)	23 µg (±14)
Clonidine	278 (25%)	199 µg (±103)
Ketamine	178 (16%)	33mg (±23)
Epidural analgesia	578 (52%)	

Epidural analgesia (with local anaesthetics and sufentanil) was used as a supplementation to general anaesthesia. Patients not receiving propofol infusion inhaled isoflurane, sevoflurane or desflurane. Data are presented as number (percentage) and mean doses administered (±SD).

Factors associated with biological cancer recurrence, univariate and multivariate analyses

Table 3 summarises univariate analyses of factors predicting BFR. Without adjusting for other variables, a pT stage of at least 3, a Gleason score of at least 7, involvement of seminal vesicles, lymph nodes, positive margin (with separate analyses for capsular incisions in pT2 or less, and positive margins in pT3 at least) and sufentanil use were associated with a statistically significant higher risk of biological recurrence ($P < 0.05$). Adjuvant radiotherapy, performed in pT2 patients with positive margins or in pT3 patients within the framework of EORTC22911 trial also significantly influenced BFR survival (b $\frac{1}{4}$ 0.80, hazard ratio 0.45, 95% confidence interval 0.18–0.72, $P \frac{1}{4}$ 0.003).

Table 3 Univariate analyses of prognostic factors and intraoperative analgesics for radical prostatectomy for cancer

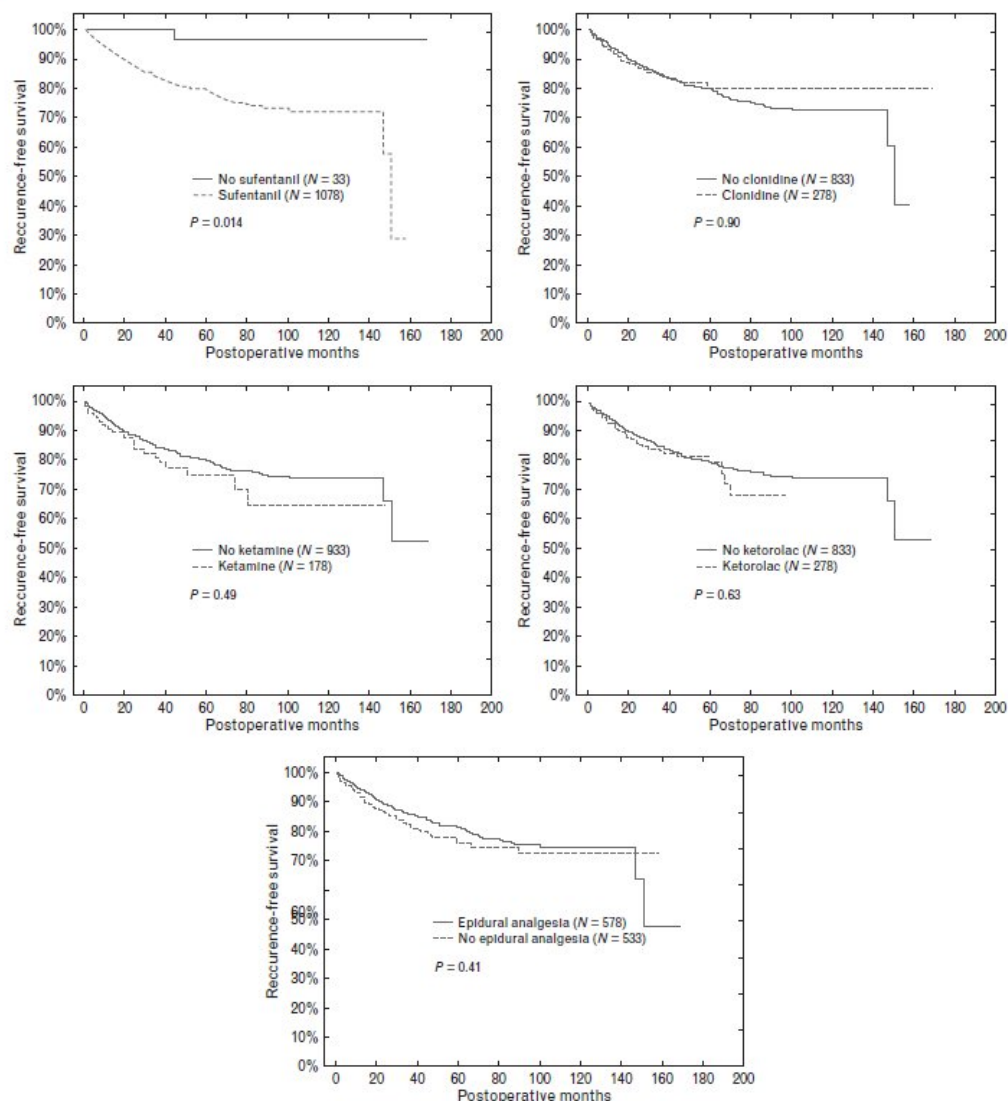
	β	HR	95% CI	P value
Preoperative PSA > 20	0.32	1.37	0.96–1.78	0.14
Extracapsular extension stage (pT $\leq$ 2 vs. pT $\geq$ 3)	–1.01	0.36	0.07–0.65	< 0.0001
Gleason score > 7	0.83	2.28	1.98–2.58	< 0.0001
Lymph node involvement	1.27	3.56	3.09–4.03	< 0.0001
Seminal vesicles involvement (no vs. yes)	–1.49	0.22	0–0.62	< 0.0001
Positive margin				
In pT1–pT4	0.96	2.62	2.35–2.89	< 0.0001
In pT $\leq$ 2	0.89	2.44	1.96–2.92	0.0003
In pT $\geq$ 3	0.55	1.74	1.38–2.10	0.003
Adjuvant androgen deprivation	0.83	2.3	0.48–4.12	0.17
Period of surgery (after 1 January 2000 vs. before)	0.21	1.24	0.67–1.53	0.16
Anaesthesia and analgesia				
Thiopental	0.032	1.03	0.54–1.52	0.90
Propofol infusion (vs. halogenated vapours)	–0.025	0.98	0.67–1.29	0.87
Ketorolac	0.098	1.10	0.70–1.50	0.63
Sufentanil	2.55	12.8	10.8–14.8	0.014
Clonidine	–0.025	0.98	0.58–1.36	0.90
Ketamine	0.15	1.16	0.64–1.58	0.49
Epidural analgesia	–0.15	0.86	0.52–1.20	0.41
Transfusion (vs. no transfusion)	–0.17	0.84	0.19–1.49	0.61

Data are presented as factor effect (β) estimated from a univariate Cox regression model, hazard ratio (HR) and associated 95% confidence interval (CI) and log-rank P value. PSA, prostate-specific antigen.

Kaplan–Meier BFR survival curves were constructed to compare patients with or without epidural analgesia and assess the impact of administration of ketorolac, sufentanil, clonidine and ketamine on the length of BFR survival (Fig. 1).

The Cox regression model was used for multivariate analysis while adjusting for all the potential confounding factors. Including the analgesics in the model and using stepwise manual backward selection for other risk factors, a pT stage of at least 3, a Gleason score of at least 7, lymph node involvement and positive margin were all retained in our multivariate analysis model (Table 4). The influence of adjuvant radiotherapy is not included in this analysis because it is outside standard treatment, but another analysis (not shown here) confirmed that our conclusions remained unaffected when it was included.

Fig. 1



Kaplan–Meier recurrence-free survival estimated for 1111 patients receiving (or not) intraoperative analgesics (sufentanil, clonidine, ketamine, ketorolac and epidural analgesia). Univariate analysis by log-rank tests. The number of patients (N) receiving or not receiving the analgesics are specified in the graphs.

Table 4 Multivariate association with cancer recurrence after radical prostatectomy

	β	HR	95% CI	P value
Extracapsular extension stage (pT $\leq$ 2 vs. pT $\geq$ 3)	-0.83	0.44	0.12–0.75	<0.0001
Gleason score $\geq$ 7	0.67	1.96	1.65–2.26	<0.0001
Positive margin (in pT1–pT4)	0.63	1.87	1.58–2.02	<0.0001
Lymph node involvement	0.57	1.77	1.27–2.27	0.024
Ketorolac	0.11	1.11	0.71–1.51	0.60
Sufentanil	2.05	7.78	5.79–9.78	0.044
Clonidine	-0.05	0.95	0.55–1.35	0.80
Ketamine	0.12	1.13	0.71–1.55	0.56
Epidural analgesia	-0.17	0.84	0.52–1.17	0.31

Association found using Cox regression model. Data are presented as factor effect (β) estimated from multivariate Cox regression model, hazard ratio (HR) and associated 95% confidence interval (CI) and P value.

DISCUSSION

Using BRC (PSA) as the primary endpoint in addition to a methodology used in a previous study in breast cancer, we have confirmed the impact of previously described risk factors such as pT stage, Gleason score, lymph node involvement and positive margin on BRF survival. We have gone further by extending the analysis to include an additional risk factor, intraoperative analgesics.^{5,15} After performing univariate and multivariate analyses, we have identified intraoperative sufentanil as a negative factor on BFR, but failed to show any statistically significant effect from epidural analgesia with local anaesthetics and opioids.

This negative effect of sufentanil confirms previous observations made in animals and humans, suggesting that synthetic opioids, such as fentanyl and sufentanil, have an undesirable influence on cancer-related immunity.¹⁸ One explanation could be that opioids modulate not only natural killer (NK) cell function but also other known immunological pathways involving monocytes, secretion of immunoglobulins, cytokines and cell proliferation.¹⁹ The effects of opioids are complex and depend on factors such as pain, age and the doses used. Small doses of morphine in painful states may be protective, although in contrast, large doses of synthetic m-agonists, especially in non-painful situations, are immunosuppressive.²¹ The peripheral mechanism for this is via a direct action on the immune cells, and centrally it is via dopaminergic pathways and liberation of neuropeptide Y.²¹

It is of interest that our current observation contrasts with an earlier analysis in breast cancer after mastectomy in which sufentanil failed to influence recurrence rate.¹⁵ Perhaps, one reason for this was that the study used only small doses of sufentanil.¹⁸ Also, studies that involve small numbers of patients must be interpreted with care.

The present analysis failed to confirm an association between epidural analgesia with local anaesthetics and sufentanil and BFR survival. This is in contrast with recently published studies suggesting a positive impact of regional analgesia, such as epidural analgesia or paravertebral blockade, on oncological outcome.^{9,10,12} These data, however, were obtained on a limited cohort of patients and were subject to flaws in the study design, chiefly the choice of endpoint and lack of data concerning intravenous analgesics used.^{9,11} The present analysis has been performed on a much larger cohort and includes all the different components of intraoperative analgesia. As NSAIDs and both intravenous and epidural opioids may influence anti-cancer immunity, it is important to include all these variables in the same analysis. In this study, the poorer outcome associated with the use of intravenous sufentanil has to be taken into account when interpreting our results concerning epidural analgesia. Indeed, if we accept that the detrimental effect of sufentanil is independent from the route of

administration, we can hypothesise that epidural sufentanil might have neutralised a potentially positive effect of epidural analgesia.

Previous studies have suggested that NSAIDs may have an impact on breast, lung, colon and PCas,^{15,20} but we did not see any effect of ketorolac on BFR survival. The impact of NSAIDs on cancers may be mediated through a direct effect on tumours over-expressing cyclooxygenase type 2, thereby influencing tumour growth, and through an indirect antagonism of prostaglandins in NK cells. Confirming this effect, however, remains a tricky challenge, considering the uneasy extrapolation from animal studies and the lack of validated perioperative immune monitoring.²¹

The α_2 -agonist clonidine potentiates hypnotics, improves postoperative analgesia and stabilises intra-operative haemodynamics. It potentially interferes with immunity through adrenergic anti-inflammatory pathways and by a central analgesic effect that reduces sympathetic tone.^{22,10,}

Ketamine is a drug widely used in the perioperative period and at sub-anaesthetic doses it prevents hyperalgesia, potentiates analgesia²⁴ and may interfere with NK activity.^{21,23} Nevertheless, we failed to find any significant effect from either of these drugs, something that is corroborated in mastectomy patients.¹⁵

The main limitation of this work is its retrospective design. Uncontrolled and unrecognised biases are frequent in retrospective studies, especially when groups are unbalanced. Indeed, the percentage of patients not receiving sufentanil here was very low (3%). The retrospective design and the very low number not receiving sufentanil together make interpretation difficult and preclude a definitive conclusion. Another possible source of bias is that the intraoperative use of sufentanil is possibly associated with the occurrence of opioid-induced hyperalgesia, increasing both pain and the need for postoperative opioids, which we did not report here. During the postoperative period, although other analgesics (paracetamol, NSAIDs) were given, we were not able to state which patients received them. This lack of information made it impossible for us to exclude any bias arising. Finally, there may be a problem with the multivariate analyses on cancer outcome, as the strength of the most powerful predictors (such as tumour extension stage) may create difficulty with identification of other subtle effects, in our case, the possible effects of perioperative analgesics.²⁵

CONCLUSION

In conclusion, these data suggest that intraoperative sufentanil may have a negative impact on biological recurrence rate after radical prostatectomy for cancer. They do not support a positive effect of epidural analgesia with local anaesthetics and opioids, but they do argue for the wider inclusion of the effects of perioperative drug administration in studies on cancer outcome following surgery. The complexity of the subject highlights the need for a validated perioperative system for monitoring the immune state to aid collation and comparison of reliable data that is complementary to prospective well conducted multicentre studies on cancer outcome.

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SECTION 3

Inflammation in cancer: Neutrophil:lymphocyte ratio and the use of ketorolac or diclofenac are prognostic factors in breast, lung and kidney cancer surgery

We observed that the ketorolac influence on cancer outcome seems to depend from the type of cancer.

This study was an opportunity to explore the place of the inflammatory response, assessed by a new, even well validated, inflammatory marker, the Neutrophil:Lymphocyte ratio. With this parameter, we show that a subgroup of patient, with a higher inflammatory response, is of higher risk of relapse.

We then investigated the effect of the non-steroidal anti-inflammatory drugs ketorolac and diclofenac in early breast and lung cancer surgery and replicated the results of our first study.

Additionally, we show why anti-inflammatory techniques are particularly promising in patients with systemic inflammation.

Neutrophil:lymphocyte ratio and intraoperative use of ketorolac or diclofenac are prognostic factors in different cohorts of patients undergoing breast, lung and kidney cancer surgery

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Keywords: Neutrophil:lymphocyte ratio, Non-steroidal anti-inflammatory drugs, cancer, surgery

Abstract

Background: Inflammation is associated with a worse outcome in cancer and Neutrophil:lymphocyte ratio (NLR) is a strong prognostic value. In cancer, non-steroidal anti-inflammatory drugs (NSAIDs) could be of interest. We investigated the prognostic significance of NLR and the impact of intraoperative NSAIDs in cancer surgeries.

Patients and methods: We performed an observational study in early breast, kidney and lung cancers (357, 227 and 255 patients) with uni- and multivariate analyses (Cox model).

Results: In breast cancer (Centre 1), a $NLR \geq 4$ is associated with a higher risk of relapse ($HR=2.41$;[95%CI:1.01-5.76], $P=0.048$). In breast cancer (Centre 2), a $NLR \geq 3$ is associated with a higher risk of relapse ($HR=4.6$;[95%CI:1.09-19.1], $P=0.04$) and higher mortality ($HR=4.0$;[95%CI:1.12-14.3], $P=0.03$). In kidney cancer, a $NLR \geq 5$ is associated with a higher risk of relapse ($HR=1.63$;[95%CI:1.00-2.66], $P=0.05$) and higher mortality ($HR=1.67$;[95%CI:1.0-2.81], $P=0.05$). In lung cancer, $NLR \geq 5$ is associated with higher mortality ($HR=1.45$;[95%CI:1.02-2.06], $P=0.04$). The intraoperative use of NSAIDs in breast cancer patients (Centre 1) is associated with a reduced recurrence rate ($HR=0.17$;[95%CI:0.04-0.43], $P=0.0002$) and a lower mortality ($HR=0.25$;[95%CI:1.08-0.75], $P=0.01$). NSAIDs use at the beginning of the surgery is independently associated with a lower metastases risk after lung cancer surgery ($HR=0.16$;[95%CI:0.04-0.63], $P=0.009$). Ketorolac use is independently associated with a longer survival ($HR=0.55$;[95%CI:0.31-0.95], $P=0.03$).

Conclusions: In these cohorts, these analyses show that NLR is a strong perioperative prognosis factor for breast, lung and kidney cancers. In this context, intraoperative NSAIDs administration could be associated with a better outcome.

Introduction

Surgery is a cornerstone in cancer treatment. Resection of a primary tumour can be a life-saving treatment, but induces an acute inflammatory response that exacerbates mechanisms linked to tumour growth and dissemination(1). These mechanisms are not completely understood. Inflammation modifies the tumoral biology itself and the quality of the immune response (1).

The inflammatory status of a patient can be assessed easily with the Neutrophil:Lymphocyte ratio (NLR). This simple parameter, associated with systemic inflammation, was initially validated by cardiologists to stratify the risk of mortality after a major cardiac event(2,3). In many cancers, including breast, kidney and lung cancers, NLR has been identified as a strong prognostic factor(4,5,6,7,8,9,10,11). Inflammatory scores have invariably been shown to be deleterious in terms of tumour progression.

To improve oncological outcome, it has been proposed to target some inflammatory mediators, notably PGE₂(1). Indeed, chronic administration of low dose aspirin has been associated with improved prevention of cancer(12,13,14,15). In the perioperative period, contrary to postoperative non-steroidal anti-inflammatory drugs (NSAIDs)(16), the benefit of a single low dose remains a matter of debate(17). In our centres some patients receive NSAIDs during the resection of a primary tumour while others do not, independently of their cancer stage(18,19). Considering that in animals a short course of NSAIDs appears sufficient to improve survival(20,21) we felt it of prime interest to question this observation in patients. Here we investigated retrospectively the effect of a single intraoperative dose of ketorolac or diclofenac during resections of primary tumours. In addition we reviewed the NLR values to validate its prognostic significance in the perioperative period of early breast, lung and kidney cancers.

Methods

Patients and methods

Ethical approval for this study (Ethical Committee N/REF 2010/15MAR/085, N°B40320108384) was provided by the IRB (CEBH of the Université catholique de Louvain, Brussels, Belgium. Chairperson Pr J.M. Maloteaux) on 29 March 2010. We reviewed four existing prospectively computed databases: two concerning breast cancer surgery, one for lung and one for kidney cancer surgeries(22,23). No power analysis was performed. Patients were treated and data collected according to the most recent guidelines (24,25,26,27,28,29,30,31,32,33). For detailed surgical and oncological data, see the appendix (text and tables A1 to A3).

Leukocytes count

Leukocytes count was typically included in the routine preoperative evaluation and prospectively registered in a computed database. The latest preoperative value was recorded. For lung cancer patients, postoperative values were available at days+1, 2 and 7 (Table A4). All venous blood samples were processed in a blood analyzer (Sysmex [TOA Medical Electronics, Kobe, Japan]) for the determination of the complete blood cell counts and differential counts of leukocytes. We recorded the neutrophils and the lymphocytes counts, and calculated the Neutrophil to Lymphocyte Ratio.

NSAIDs administration

Intraoperative use of ketorolac (typically 20 mg in patients under 60 kg, and 30 mg in patients over 60 kg) or diclofenac (75 mg), and timing of administration (at the beginning or the end of surgery) were recorded. The NSAIDs choice and time of administration were at the anaesthesiologist's discretion according to contraindications (renal, gastrointestinal). All surgeries were performed under general anesthesia.

Endpoints

Our primary objectives were to confirm the prognostic significance of preoperative NLR value, and to investigate the effect on outcome (recurrence rate and overall survival) of intraoperative NSAIDs (ketorolac or diclofenac). The impact of the timing of administration (at the beginning or end of the surgical procedure) was also investigated. Consequently, our primary endpoints were overall survival time(34) and length of recurrence-free survival. Locoregional recurrences were analyzed separately for lung cancer(35). For detailed

endpoints definitions, see the appendix.

Statistical analysis

Patients' baseline characteristics are presented as mean $\pm$ SD or number (percentage). Follow-up and survival lengths are presented as median [interquartile 25-75] [IQR] or number (percentage) [95% confidence interval, CI]. Recurrence-free and overall survival rates are presented at fixed time points (24 and 60 months) (excepted for breast cancer patients in Centre 2 where it is possible only at 24 months). Categorical variables were compared with Chi-square test, and continuous ones with Student t-test. Univariate Cox model and logrank test were used to assess the potential impact of these baseline characteristics and to investigate the prognostic value of NLR on outcome. Kaplan-Meier analyses were used to estimate recurrence-free survival and overall survival probabilities. Assuming the possibility of a non-linear impact of the NLR-value on outcome, violating the Cox model's assumption, we introduce it as a binary variable ($NLR \geq 5$ or $NLR < 5$) as proposed by Proctor *et al*(11). To avoid spurious conclusions, we compared only groups of at least 30 patients. As a consequence, if the number of patients with a $NLR \geq 5$ was < 30 , the analysis was not performed. If the analysis was possible with a cut-off value of 4 or 3 (i.e. with $N > 30$ in all the groups), the highest value was chosen. After univariate analysis, Cox regression model was used for multivariate analysis while adjusting for any baseline factors and intraoperative or oncological factors related with the outcome in the univariate analysis ($P \leq 0.05$). We used stepwise manual backward regression and all factors significant at P value ≤ 0.05 were retained in the final model. To lower the risk of overfitting, only Cox models for which the number of outcome events was at least 10 per independent variable were considered(36). STATISTICA (data analysis software system) version 7 (Statsoft Inc. 2004, Tulsa, USA) was used for all analyses.

Results

Breast cancer patients: Centre 1

Summary of the results in breast cancer patients in Centre 1: $NLR \geq 4$ and NSAID use are prognostic factor of recurrence-free survival in uni- and multivariate analyses. Univariate analyses argues for their prognostic value in risk of death.

The data from 172 patients meeting the inclusion criteria were reviewed (Appendix: text and table A1). Briefly, to summarize inclusion criteria, we included: patients with mastectomy and axillary clearance without history of neoadjuvant chemotherapy, previous ipsilateral and/or non curative surgery. Ketorolac at the beginning of the surgical procedure was the most frequent NSAID administration. Because too few patients received diclofenac, the data for ketorolac and diclofenac were pooled for subsequent analyses.

Preoperative leukocytes counts.

In the whole series, the median NLR was 2.6[IQR: 1.9-3.9]. Only 20 patients had a $NLR \geq 5$. According to our methodology, the chosen cut-off value was 4 in order to compare groups with $N > 30$.

Association between NLR and NSAIDs with cancer recurrence in univariate and multivariate analyses.

The risk of recurrence was significantly higher when the NLR was ≥ 4 vs < 4 (Recurrence rate at 24 months: 16.5%[95%CI: 4.4-28.5] vs 6.3%[95%CI: 2.1-10.5]; at 60 months: 24.5%[95%CI: 10-39] vs 10.6%[95%CI: 5-16.2], $P=0.03$).

Univariate analysis showed that $NLR \geq 4$, age and lymph node invasion were significantly associated with shorter recurrence-free survival (Table 1, figure A1). Using a Cox regression model with stepwise manual backward selection, histological grade ($HR=3.29$; [95%CI: 1.26-8.59], $P=0.015$) and $NLR \geq 4$ ($HR=2.46$; [95%CI: 1.03-5.89], $P=0.04$) were retained in our multivariate model.

A significantly lower risk of recurrence was observed when NSAIDs were used (Recurrence rate at 24 months: 2.2%[95%CI: 0-5] vs 21.8%[95%CI: 10.9-32.8]; at 60 months: 6.9%[95%CI: 1.9-11.9] vs 29.6%[95%CI: 17-42.2], $P=0.00003$)(Figure 1). In multivariate analysis, NSAID use was a strong predictor of recurrence-free survival ($HR=0.17$; [95%CI: 0.07-0.43], $P=0.0002$)(Table 1).

Association between NLR and NSAIDs with mortality risk in univariate analysis.

Risk of death was not different for patients with $NLR \geq 4$ vs < 4 (Mortality at 24 months:

8.2%[95%CI:0-17.1] vs 2.4%[95%CI:0-5]; at 60 months: 16.9%[95%CI:3.7-30.1] vs 8.2%[95%CI:3.1-13.2], $P>0.05$)(Table 1, figure A2).

Risk of death was lower for patients receiving NSAID than for those who did not (Mortality at 24 months: 1.8%[95%CI:0-4.2] vs 8.9%[95%CI:1.4-16.3]; at 60 months: 5.5%[95%CI:1-10] vs 20.7%[95%CI:9.3-32], $P=0.009$)(Figure 2). NSAID use was associated with a lower risk of death (HR=0.25;[95%CI:0.08-0.75], $P=0.01$).

Because of too few events (death), multivariate analysis on mortality was not performed(36).

Breast cancer patients: Centre 2

Summary of the results in breast cancer patients in Centre 2: NLR $\geq$ 3 is a prognostic factor of recurrence-free survival and risk of death in univariate analyses..

The data from 162 patients meeting the inclusion criteria were analysed (Appendix: text and table A1). Inclusion criteria were: patients with mastectomy or conservative surgery, both with axillary clearance, and without history of neoadjuvant chemotherapy, previous ipsilateral and/or non curative surgery As in centre 1, except for age all the patients and tumours' characteristics were similar between the patients receiving or not a NSAID. Only ketorolac was used, mostly at the beginning of the surgical procedure.

Preoperative leukocytes counts.

In Centre 2, indication for leukocyte counts was different than in Centre 1 (only high risk patients in Centre 1, all patients in Centre 2). The median NLR was 2.1[IQR:1.6-3.4]. Only 13 patients had a NLR $\geq$ 5. According to our methodology, the cut-off value chosen was 3.

Association between NLR and ketorolac with cancer recurrence in univariate analysis.

The risk of recurrence was significantly higher when the NLR was $\geq$ 3 (Recurrence rate at 24 months: 11.6%[95%CI:0.7-22.5] vs 2.2%[95%CI:0-5.1], $P=0.01$)(Appendix: Figure A3).

Univariate analysis showed that histological grade and NLR $\geq$ 3 were significantly associated with shorter recurrence-free survival (Table 1).

A non-statistically significant lower recurrence risk was observed when ketorolac was used (Recurrence rate at 24 months: 3%[95%CI:0-7.1] vs 6.6%[95%CI:0.3-13], $P=0.20$)(Figure 3).

Risk reduction for recurrence was not statistically significant in Cox model (HR=0.38;[95%CI:0.07-1.95], $P=0.24$).

Because of too few events, multivariate analysis was not possible (36).

Association between NLR and ketorolac with mortality risk in univariate analysis.

The risk of death was higher when the NLR was ≥ 3 vs < 3 (Mortality at 24 months: 10.1%[95%CI:0.6-19.7] vs 3.1%[95%CI:0-6.5], Logrank test: $P=0.04$). $NLR \geq 3$ was associated with mortality in univariate analysis (Table 1).

Because of too few events, multivariate analysis on recurrence rate and mortality were not possible(36).

A non-statistically significant different risk of death was observed in patients receiving ketorolac intraoperatively (Mortality at 24 months: 3.4%[95%CI:0-7.5] vs 7.9%[95%CI:1.2-14.5], $P=0.23$)(Figure 4). In Cox model also, the lower risk of death was not statistically significant (HR=0.44;[95%CI:0.11-1.77], $P=0.25$)(Table 1). According to our methodology, we did not perform a multivariate analysis.

NSCLC patients

Summary of the results in NSCLC patients: $NLR \geq 5$ is an independent prognostic factor of distant metastasis-free survival and a prognostic factor of mortality in univariate analyses. NSAID use is a prognostic factor both for distant metastasis-free survival (particularly ketorolac and given at the beginning of surgery) and risk of death, in uni- and in multivariate analyses.

Patients and procedures.

The data from 255 patients meeting the inclusion criteria were reviewed (Appendix: text and table A2). Inclusion criteria were: patients undergoing curative-intended resection for primary stage I or II NSCLC with free macro- and microscopic margins. Sixty-six patients received a NSAID during surgery (26%). Ketorolac was administered more frequently (66%) than diclofenac, and NSAIDs were more frequently administered at the beginning of the surgical procedure (65%)(Table A2).

Preoperative and postoperative leukocytes counts.

The median NLR was 3.4[IQR:2.3-4.9]. Sixty-four patients had a $NLR \geq 5$. Neutrophils counts and NLR were significantly higher, whereas lymphocytes counts were significantly lower at day+1, day+2 and day+7 after surgery, as compared with the preoperative values (Paired t-test) (Table A4). These differences between pre- and postoperative NLR values were still present when separating survivors from non-survivors. For the survivors NLR returned to preoperative values more quickly than for the non-survivors (Figure A5).

Associations between NLR, NSAIDs and risk of distant metastasis but not of locoregional recurrence

A tendency toward a higher risk of distant metastasis occurrence was observed when the preoperative NLR was ≥ 5 vs < 5 (Distant metastasis rate at 24 months: 22.1%[95%CI:11-33.2] vs 10.7%[95%CI:6-15.3]; at 60 months: 31%[95%CI:17.8-44.3] vs 20.4%[95%CI:13.9-26.9], $P=0.08$) (Figure A5). Other risk factors for occurrence of distant metastasis were node status, tumor size > 20 mm and $NLR \geq 5$ (Table 2).

Stage II was the only risk factor associated with a higher risk of locoregional recurrence. No other correlation was seen with the rate of locoregional recurrences (Table 2).

Concerning NSAIDs use, a tendency was also observed toward a lower risk of distant metastases occurrence when the patients received a NSAID intraoperatively vs no NSAID (Distant metastasis rate at 24 months: 10%[95%CI:2.4-17.6] vs 14.7%[95%CI:9.2-20.2]); at 60 months: 14.9%[95%CI:5.6-24.2] vs 26.3%[95%CI:18.9-33.7], $P=0.07$) (Tables 2, 4; figure 5).

A longer metastasis-free survival was observed after the use of NSAIDs (Table 2).

Risk factors for recurrence. Multivariate analyses.

Cox regression model was used for multivariate analysis while adjusting for these factors and all the risk factors described in Table 2(23). Using stepwise manual backward selection, Cox regression model showed that NSAID use, node status and $NLR \geq 5$ were independent risk factors of distant metastasis-free survival ($P < 0.05$)(Table 3). Because of variables colinearity, the effect of the time of administration of NSAIDs (at the beginning vs at the end of surgery) was tested in a second multivariate model. In this model, node status and $NLR \geq 5$ remain independent risk factors of distant metastasis-free survival ($P < 0.05$). NSAIDs use at the beginning of the surgery compared with no NSAIDs was associated with a better distant-metastasis-free survival (HR:0.16;[95%CI:0.04-0.63], $P=0.009$).

Association between NLR and NSAIDs with mortality risk in univariate analyses.

The risk of death was higher when the NLR was ≥ 5 vs < 5 (Mortality at 24 months: 34.4%[95%CI:22.7-46] vs 17.7%[95%CI:12.2-23.2]; at 60 months: 56.8%[95%CI:44.5-69] vs 38.3%[95%CI:31.1-45.5], $P=0.005$)(Figure A6).

The risk of death was lower in patients receiving a NSAIDs intraoperatively (Mortality at 24 months: 13.6%[95%CI:5.4-21.9] vs 25%[95%CI:18.7-31.2]; at 60 months: 34.6%[95%CI:22.8-46.3] vs 46.2%[95%CI:38.8-53.6], $P=0.028$)(Figure 6). Other risk factors for mortality were pneumonectomy and tumor size > 20 mm ($P < 0.05$)(Table 2).

Risk factors for mortality. Multivariate analyses.

In multivariate Cox regression model, pneumonectomy and ketorolac were retained as

independent prognostic factors ($P<0.05$)(Table 3).

Kidney cancer patients

Summary of the results in kidney cancer patients: $NLR \geq 5$ is a prognostic factor of cancer recurrence and risk of death in uni- and multivariate analyses.

Patients and procedures, histopathological findings.

Data from 227 patients were reviewed (Appendix: text and table A3). Inclusion criteria were: patients undergoing curative-intended resection of a primary kidney tumour. Only 19 patients received an NSAID intraoperatively (ketorolac: N=13; diclofenac: N=6) precluding any analysis on the NSAIDs' impact.

Association between NLR and cancer recurrence in uni- and multivariate analyses.

The median NLR was 3.01[IQR:1.97-4.49]. Fifty two patients had a $NLR \geq 5$.

The risk of recurrence was significantly higher when the NLR was ≥ 5 vs < 5 (Recurrence rate at 24 months: 32.9%[95%CI:19.7-46.2] vs 21%[95%CI:14.6-27.4]; at 60 months: 47.8%[95%CI:33-62.6] vs 31.5%[95%CI:23.8-39.2], $P=0.03$) (Figure A7). Univariate analysis showed that age, node status, stage and histological grade were significantly associated with shorter recurrence-free survival (Table 4). The recurrence rate was slightly higher when NLR was ≥ 5 (Figure A7). Cox regression model was used for multivariate analysis while adjusting for all the potential influencing factors described above. Using stepwise manual backward selection, node status (HR=3.3;[95%CI:1.88-5.86], $P<0.0001$) and stage (All vs I) (HR=1.3;[95%CI:1.16-1.47], $P<0.0001$) were retained as independent risk factors for metastasis occurrence (Table 7).

Association between NLR and mortality in uni- and multivariate analysis.

The risk of death was higher when the NLR was ≥ 5 vs < 5 (Mortality at 24 months: 29%[95%CI:16.2-41.8] vs 12.5%[95%CI:7.4-17.6]; at 60 months: 40%[95%CI:26-54] vs 24.8%[95%CI:17.9-31.7], $P=0.02$). Age, node status, stage, histological grade and $NLR \geq 5$ were associated with mortality in univariate analysis.

In multivariate analysis, node status (HR=2.55;[95%CI:1.48-4.43], $P=0.0008$), stage (All vs I) (HR=1.66;[95%CI:1.03-1.31], $P<0.0001$), histological grade and $NLR \geq 5$ (HR=1.67;[95%CI:1.0-2.81]; $P=0.05$) were retained in the model as independent prognostic factors of mortality.

Discussion

The first result of this study is the prognostic value of preoperative NLR in patients with breast, lung and kidney cancers undergoing surgery for their primary tumour. The strong and independent value of NLR as a prognostic factor for cancer recurrence and for postoperative mortality confirms and extends the results obtained by Azab *et al* in patients with breast cancer. In that study, NLR levels prior to chemotherapy predicted short and long-term mortality, and were correlated with the tumour's size and patient's age(9). In our results, NLR was associated with recurrence-free survival and mortality independently from tumour's size and patient's age.

For patients with breast, lung and kidney cancer our results suggest that NLR could help to identify high risk groups as early as before surgery for the primary tumour. Possible explanations for the prognostic value of a systemic inflammatory marker are the numerous links between inflammation and carcinogenesis(1). More specifically, which cancer-promoting factors could be increased in patients with more neutrophils or less lymphocytes? Tumour-associated neutrophils have been implicated in the production of reactive oxygen species (ROS), basic fibroblast growth factor (bGF), PGE2, and vascular endothelial growth factor (VEGF), leading to more aggressive tumours(37,38,39). PGE2 directly decreases the number and activity of Natural Killer cells, by modifying the intracellular level of cyclic adenosine monophosphate(40,41,42,43). Moreover it indirectly increases the proportions of immunosuppressive macrophages or myeloid-derived suppressor cells within tumours(44,45). Many other inflammatory soluble factors such as IL1- β and IL-6 have also been shown to promote survival, proliferation or migration of tumour cells and could be proposed(1).

The other result of our study was the association between the intraoperative administration of NSAIDs (ketorolac or diclofenac) with a longer recurrence-free survival in breast cancer patients, and with better distant metastasis-free and overall survival in NSCLC patients (especially when given at the beginning of the surgery). It is worth noting that the validation of the observation made in breast cancer patients in Centre 1 was not possible in Centre 2, because of different proportions of grade 3 tumours impacting both the NLR and, not surprisingly, the outcome(46) (for details, see the appendix).

Considering that NSAID are routinely administered to almost all patients in the postoperative period, it is tempting to speculate that intraoperative NSAIDs could have an effect on the dissemination of tumour cells during surgery, an effect that would require the presence of

NSAIDs during a short period of time that includes and follows the surgical manipulation of the primary tumour. In patients with prostate cancer, we did not observe a prognostic value for the intraoperative administration of ketorolac during prostatectomy(47). Therefore, the effect could be cancer specific (including tumour's and host's response characteristics).

NSAIDs have been reported to reduce tumour cell adhesion to endothelial cells(48) and to inhibit tumour cell invasion(49,50). If NSAIDs inhibit the release of tumour cells into the blood, it would be interesting to compare the levels of circulating tumour cells during or immediately after surgery in patients who did or did not receive the drug. But NSAIDs could also decrease the capacity of the circulating tumour cells to enter into their metastatic location, or to survive in this new microenvironment, and in these cases the levels of circulating tumour cells will not change. Finally, hypothesizing that the NSAIDs beneficial effect would be greater in patients with higher inflammatory response (involving similar pathways), we explored the NSAIDs effect in breast cancer patients with a NLR $\geq$ 4. We observed a 2-fold higher reduction of the risk of recurrence with NSAIDs (Figure 8). This result is however based on very few patients and needs to be confirmed in another study.

We are well aware of the methodological limitations of our retrospective analyses of prospectively collected data. These analyses focused on heterogeneous, even if selected, populations. We did not exclude patients on steroid therapy and patients with a possible infectious process (typically in patients with chronic obstructive pulmonary disease). But even considering the same parameters (i.e. NLR and NSAIDs) in these different and heterogeneous populations, our results are conclusive. Nevertheless, we cannot definitively exclude an unrecognized bias.

To conclude, our results suggest that, at least in these cohorts, perioperative NLR might be an interesting parameter for breast, lung and kidney cancer patients undergoing surgical resection of their primary tumor. Moreover, intraoperative administration of NSAID is an extremely simple medical act that appears to be associated with a better prognosis for these patients. These observations warrant prospective clinical studies.

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Tables

Table 1. Univariate analyses of possible risk factors of recurrence in patients operated for breast cancer surgery in Centre 1 and 2. Data are presented as factor effect (beta) estimated from univariate Cox regression model, hazard ratio (HR) and associated 95%CI and *P*-value.

	Beta	HR	95% CI	<i>P</i> value	Beta	HR	95% CI	<i>P</i> value
Centre 1 (N=172)	Recurrence risk				Mortality risk			
Tumour size	-0.003	1	0.98-1.02	0.78	-0.03	0.97	0.93-1.01	0.08
Histological grade	1.35	3.87	1.48-10.07	0.01	1.28	3.61	0.73-9.87	0.11
Lymph node invasion	0.52	1.68	0.95-2.94	0.07	0.54	1.72	0.84-3.53	0.14
Nottingham Prognostic Index	-0.01	0.99	0.9-1.09	0.83	-0.01	0.99	0.88-1.12	0.84
Hormonal receptor status								
Estrogen positive	-1.27	0.28	0.09-0.86	0.02	-1.04	0.35	0.08-1.52	0.15
Progesteron positive	1.27	3.58	0.64-19.9	0.14	0.78	2.17	0.35-13.46	0.39
HER-2 expression	0.12	1.13	0.47-2.7	0.78	0.97	2.64	0.81-8.58	0.11
NLR$\geq$4 (vs <4)	0.99	2.68	1.08-6.62	0.03	0.54	0.51	0.35-8.58	0.47
Ketorolac or diclofenac use	-1.81	0.16	0.06-0.42	0.0002	-1.39	0.25	0.08-0.75	0.01
Centre 2 (N=162)	Recurrence risk				Mortality risk			
Total mastectomy	0.13	1.13	0.25-5.25	0.87	0.08	1.08	0.29-4.04	0.90
Tumor size	0.004	1.004	0.98-1.02	0.75	0.01	1.01	0.99-1.03	0.11
Histological grade	1.8	6.28	1.31-27.9	0.02	0.87	2.40	0.88-6.49	0.09
Lymph node invasion	0.61	1.85	0.05-69.1	0.21	0.41	1.51	0.73-3.11	0.26
Nottingham Prognostic Index	0.02	1.02	0.91-1.14	0.72	-0.03	0.97	0.86-1.09	0.64
Hormonal receptor status								
Estrogen positive	-0.88	0.41	0.10-1.73	0.23	0.83	2.30	0.28-18.7	0.43
Progesteron positive	-0.47	0.62	0.14-2.83	0.54	0.70	2.02	0.42-9.66	0.38
HER-2 expression	0.20	1.22	0.27-5.52	0.80	2.05	7.75	0.97-62.0	0.056
NLR$\geq$3 (vs <3)	1.52	4.60	1.09-19.1	0.04	1.39	4.00	1.12-14.3	0.03
Ketorolac use	-0.98	0.38	0.07-1.95	0.24	-0.82	0.44	0.11-1.77	0.25

Table 2. Univariate analysis of possible prognostic factors for distant metastases occurrences in patients undergoing lung surgery for non-small cells lung cancer. Distant metastases are defined as clinical evidences of distant metastase(s) development (out of the operated hemithorax), confirmed by radiological examination(s), and locoregional recurrences as evidences of recurrence(s) in the ipsilateral lung or mediastinum (23,35). Data are presented as factor effect (beta) estimated from univariate Cox regression model, hazard ratio (HR) and associated 95%CI and P-value. NSAID: Non-steroidal anti-inflammatory drug.

	Beta	HR	95% CI	P-value	Beta	HR	95% CI	P-value	Beta	HR	95% CI	P-value
N=255	Distant metastases occurrence risk				Locoregional recurrence risk				Mortality risk			
Pneumonectomy	0.21	1.23	0.65-2.36	0.54	0.29	0.59	0.65-2.76	0.44	0.80	2.23	1.50-3.29	<0.001
Node status	0.38	1.46	1.07-2.00	0.02	0.34	1.40	0.99-2.00	0.07	0.06	1.06	0.82-1.37	0.67
Grade of differentiation (poor/moderate)	0.15	1.16	0.60-2.26	0.66	0.02	1.02	0.48-2.19	0.95	-0.08	0.92	0.60-1.42	0.71
Size greater than 20 mm	0.68	1.97	1.14-3.42	0.02	0.42	1.52	0.85-2.74	0.17	0.42	1.52	1.09-2.12	0.01
Stage (II vs I)	0.86	2.35	1.37-4.09	0.002	1.26	3.51	1.85-6.73	<0.001	0.49	1.63	1.15-2.32	0.007
Histology (Adenocarcinoma vs squamous)	-0.03	0.97	0.62-1.52	0.91	0.18	1.19	0.73-1.95	0.48	-0.07	0.93	0.71-1.23	0.62
NLR (≥ 5 vs < 5)	0.61	1.84	1.04-3.25	0.03	0.08	1.08	0.51-2.28	0.83	0.42	1.52	1.07-2.17	0.02
NSAID use	-0.70	0.50	0.24-1.00	0.05	0.07	1.07	0.54-2.13	0.84	-0.45	0.64	0.42-0.96	0.03
Ketorolac (vs no NSAID)	-0.55	0.58	0.26-1.29	0.17	-0.04	0.97	0.42-2.19	0.93	-0.90	0.41	0.23-0.70	0.001
Diclofenac (vs no NSAID)	-1.99	0.14	0.02-0.99	0.05	-0.40	0.67	0.20-2.22	0.51	-0.49	0.61	0.35-1.06	0.08
NSAID use at the beginning of the surgery	-1.75	0.17	0.03-0.87	0.03	0.18	1.20	0.31-4.63	0.79	-0.08	0.92	0.42-2.02	0.83

Table 3. Multivariate association with distant metastases occurrence and mortality after lung surgery for non-small cell lung cancer: Cox regression model (N=255). Data are presented as factor effect (beta) estimated from multivariate Cox regression model, hazard ratio (HR) and P-value. NSAID: Non-steroidal anti-inflammatory drug.

N=255	Beta	HR	95% CI	P-value
Distant Metastases occurrence risk				
Node status	0.43	1.54	1.15-2.06	0.005
NLR (≥ 5 vs < 5)	0.58	1.78	1.0-3.19	0.05
NSAID	-0.74	0.48	0.23-0.98	0.04
Mortality risk				
Pneumonectomy	0.76	2.14	1.45-3.15	0.0001
Ketorolac (vs no NSAID)	-0.61	0.55	0.31-0.95	0.03

Table 4. Univariate analysis of possible prognostic factors for recurrence in patients undergoing nephrectomy for kidney cancer. Data are presented as factor effect (beta) estimated from univariate Cox regression model, hazard ratio (HR) and associated 95%CI and P-value.

	Beta	HR	95% CI	P-value	Beta	HR	95% CI	P-value
N=227	Metastases occurrence risk				Mortality			
Age	0.022	1.02	1.0-1.05	0.05	0.037	1.04	1.02-1.06	0.03
Sex (Male vs female)	0.38	1.46	0.84-2.53	0.17	0.47	1.6	0.92-2.77	0.09
Node status	1.37	3.95	2.10-7.37	<0.0001	1.42	4.1	2.12-8.06	<0.0001
Histological grade	0.58	1.78	1.26-2.54	0.001	0.69	1.99	1.37-2.89	0.0002
Stage (All vs I)	0.25	1.28	1.14-1.44	<0.0001	0.29	1.22	1.21-1.47	0.0002
NLR (> 5 vs < 5)	0.45	1.56	0.94-2.61	0.078	0.49	1.63	1.00-2.66	0.05

Table A1. Characteristics of patients undergoing breast cancer surgery in two independent centres and following the intraoperative use the non-steroidal anti-inflammatory drugs (NSAIDs) ketorolac or diclofenac. Data are presented as mean +/- SD or as number (percentage). * $P < 0.05$ compared with No NSAID. NLR: Preoperative Neutrophil:Lymphocyte ratio.

	Centre 1 (N=172)		Centre 2 (N=162)	
	No NSAID (N=60)	NSAIDs use (N=112)	No NSAID (N=80)	NSAIDs use (N=82)
Age (years)	67+/-13	56+/-13 *	64+/-13	59+/-10 *
Total mastectomy	60 (100%)	112 (100%)	40 (50%)	46 (56%)
Tumour size (mm)	33+/-17	35+/-23	36+/-25	33+/-26
Histological grade				
1	4 (7%)	12 (11%)	13 (16%)	13 (16%)
2	23 (38%)	48 (43%)	39 (49%)	43 (52%)
3	33 (55%)	52 (46%)	28 (35%)	26 (32%)
Lymph node invasion				
1 (none)	27 (45%)	66 (59%)	42 (53%)	36 (44%)
2 (1-3 positive lymph nodes)	19 (32%)	30 (27%)	19 (24%)	22 (27%)
3 (>3 positive lymph nodes)	14 (23%)	16 (14%)	19 (24%)	24 (29%)
Nottingham Prognostic Index	10.8+/-3.9	10.8+/-4.9	9.3+/-5.1	9.5+/-5.8
Hormonal receptor status				
Estrogen positive	50 (83%)	95 (85%)	62 (78%)	64 (78%)
Progesteron positive	50 (83%)	103 (92%)	52 (65%)	59 (72%)
HER-2 expression	25 (42%)	56 (50%)	29 (36%)	44 (54%)
NLR	3.05+/-1.98	3.42+/-3.38	2.66+/-1.72	2.61+/-1.42
Ketorolac use	-	91 (81%)	-	82 (100%)
At the beginning of the surgery	-	82 (90%)	-	99 (88%)
Diclofenac use	-	21 (19%)	-	0
At the beginning of the surgery	-	19 (90%)	-	-

Table A2. Characteristics of patients undergoing lung cancer surgery following the intraoperative use the non-steroidal anti-inflammatory drugs (NSAIDs) ketorolac or diclofenac. Data are presented as mean +/- SD or as number (percentage). $P>0.05$ comparing NSAIDs use with No NSAID. NLR: Preoperative Neutrophil:Lymphocyte ratio (N=255).

	No NSAID (N=189)	NSAIDs use (N=66)
Age (years)	64.4 +/- 9.1	63.5 +/- 16.3
Males / females	158 (84%) / 31 (26%)	52 (79%) / 14 (21%)
Thoracoscroe	0.077 +/- 0.061	0.061 +/- 0.031
Pathological stage		
I	144 (76%)	50 (76%)
II	44 (23%)	16 (24%)
Surgical resection		
Pneumonectomy	36 (19%)	7 (11%)
Lobectomy/bilobectomy	149 (79%)	57 (86%)
Segmentectomy/wedge	5 (3%)	1 (2%)
Histology		
Squamous	99 (52%)	37 (56%)
Adenocarcinoma	82 (43%)	31 (47%)
Undifferentiated carcinoma	6 (3%)	0
Tumor size		
< 20 mm	97 (51%)	27 (41%)
> 20 mm	92 (49%)	39 (59%)
Lymph node invasion (Node positive)	149 (79%)	53 (80%)
NLR	4.15 +/- 3.96	3.70 +/- 1.55
Ketorolac use	-	43 (66%)
At the beginning of the surgery	-	28 (65%)
Diclofenac use	-	23 (35%)
At the beginning of the surgery	-	15 (65%)

Table A3. Characteristics of patients undergoing nephrectomy for kidney cancer. Data are presented as mean +/- SD or as number of patients (percentage) (N=227).

Patients and histological findings	
Age (years)	63+/-12
Males / females	71 (31%) / 156 (69%)
Pathological stage	
I	74 (33%)
II	91 (40%)
IIIa	23 (10%)
IIIb	24 (11%)
IIIc	11 (5%)
IV	4 (2%)
Histological grade	
1	78 (34%)
2	119 (52%)
3	30 (13%)
Histology	
Clear cell	166 (73%)
Tubulo-papillary carcinoma	29 (13%)
Chromophobe	4 (2%)
Other	28 (12%)
Tumor size	
< 40 mm	43 (19%)
40-69 mm	53 (23%)
70-99 mm	75 (33%)
> 99 mm	32 (14%)
Unknown	24 (11%)
Lymph node invasion (Node positive)	13 (6%)

Table A4. Leukocytes, neutrophil:lymphocyte ratio (NLR) and platelet counts one or two days before surgery and in the postoperative week, in patients undergoing lung resection for primary NSCLC. Data are presented as mean $\pm$ -SD and median [interquartile range 27-75] (N=255). Data are compared vs baseline (preoperative value) using paired t-tests.

Leukocytes counts (N=255)	Mean $\pm$ -SD	Median [IQR 25-75]	P-value vs baseline
Neutrophils (per μL)			
Preoperative	5.5 $\pm$ 2.2	5.2 [4-6.6]	-
Day +1	9.1 $\pm$ 3.1	8.8 [7.2-10.8]	<0.001
Day +2	9.0 $\pm$ 3.8	8.2 [6.5-11]	<0.001
Day +7	7.0 $\pm$ 3.2	6.2 [4.9-8.4]	<0.001
Lymphocytes (per μL)			
Preoperative	1.7 $\pm$ 0.7	1.6 [1.2-2]	-
Day +1	1.1 $\pm$ 0.6	1.0 [0.7-1.2]	<0.001
Day +2	1.2 $\pm$ 0.6	1.2 [0.8-1.5]	<0.001
Day +7	1.4 $\pm$ 0.7	1.4 [0.9-1.8]	<0.001
NLR			
Preoperative	4.0 $\pm$ 3.5	3.4 [2.3-4.9]	-
Day +1	11.4 $\pm$ 10.3	9.0 [6.2-14]	<0.001
Day +2	9.1 $\pm$ 7.0	7.2 [4.9-11.7]	<0.001
Day +7	7.0 $\pm$ 9.0	4.4 [3.1-7.1]	<0.001

Figures

Figure 1. Kaplan-Meier curves of recurrence-free survival for 172 patients receiving or not an intraoperative administration of ketorolac or diclofenac during resection of a primary breast tumour in Centre 1. Univariate analysis by logrank test.

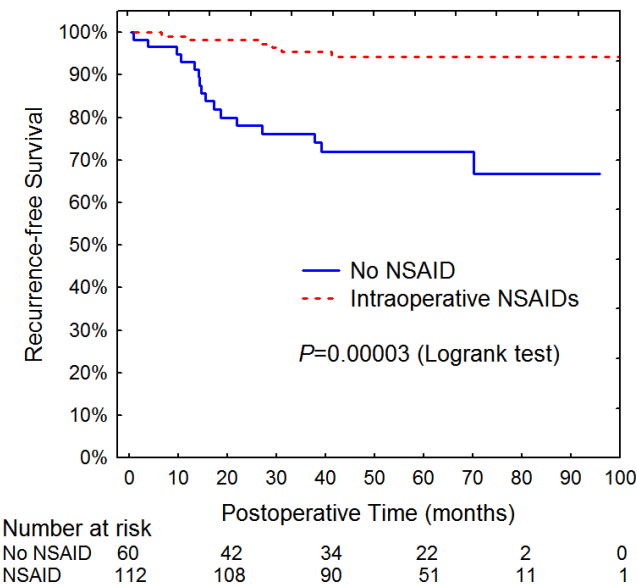


Figure 2. Kaplan-Meier curves of overall survival for 172 patients receiving or not receiving an intraoperative administration of ketorolac or diclofenac during resection of a primary breast tumour in Centre 1. Univariate analysis by logrank test.

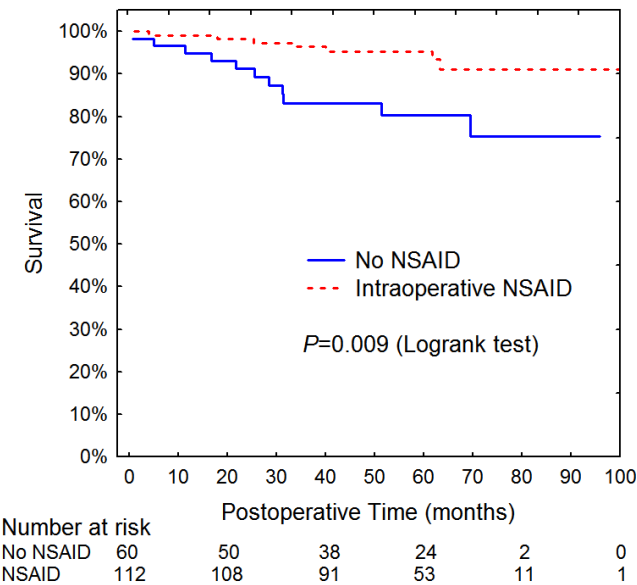


Figure 3. Kaplan-Meier curves of recurrence-free survival for 162 patients receiving or not receiving an intraoperative administration of ketorolac during resection of a primary breast tumour in Centre 2. Univariate analysis by logrank test.

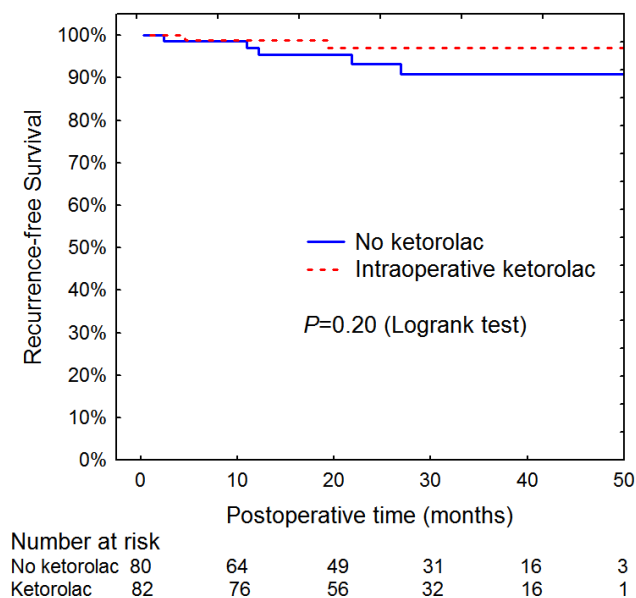


Figure 4. Kaplan-Meier curves of overall survival for 162 patients receiving or not receiving intraoperative ketorolac for a breast cancer surgery in Centre 2. Univariate analysis by logrank test.

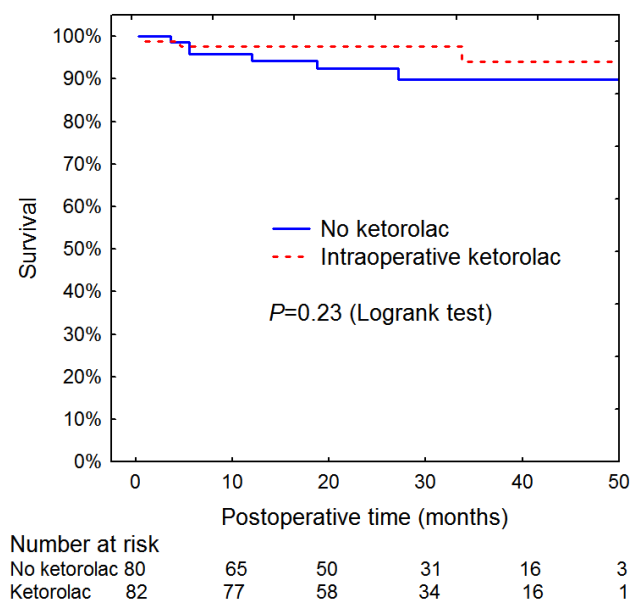


Figure 5. Neutrophil:lymphocyte ratio (mean $\pm$ SEM) in patients undergoing surgery for NSCLC, surviving or not during the follow-up period. * $P < 0.05$ comparing survivors vs non survivors at the same postoperative day (Student t-tests). ** $P < 0.001$ comparing, in each group (survivors and non survivors), the value at postoperative day +1, +2 and +7 vs the preoperative value (paired t-test). $P = 0.088$ comparing the preoperative values (survivors vs non survivors).

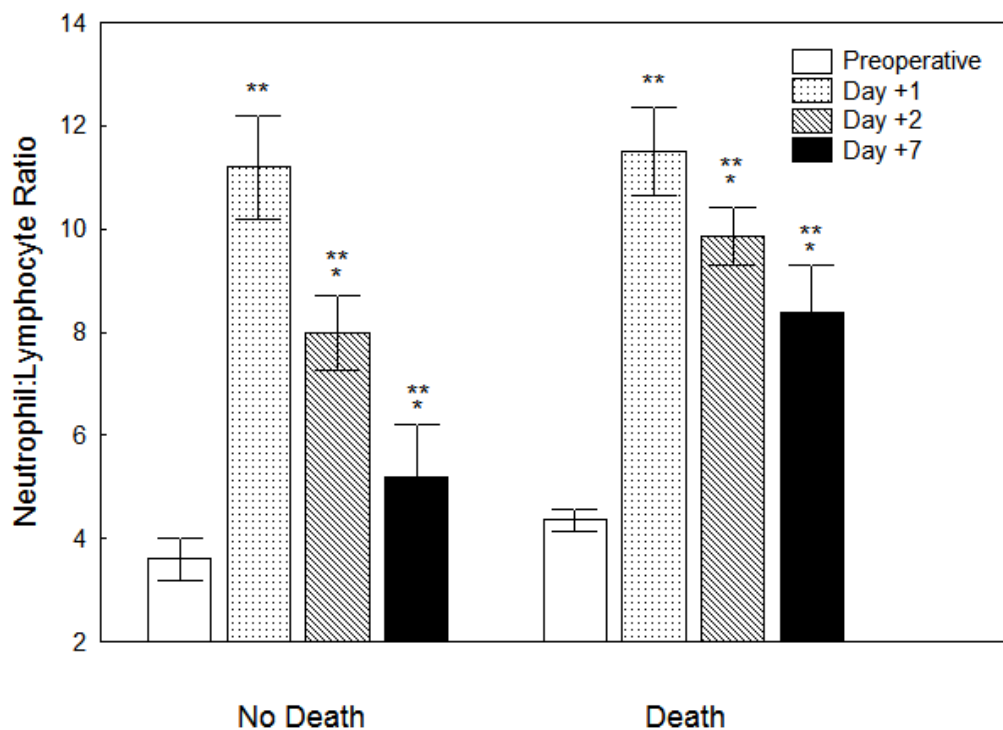


Figure 6. Kaplan-Meier curves of distant metastasis-free survival for 255 patients receiving or not intraoperatively a non-steroidal anti-inflammatory drug (NSAID), ketorolac or diclofenac during lung surgery for primary NSCLC. Univariate analysis by logrank test.

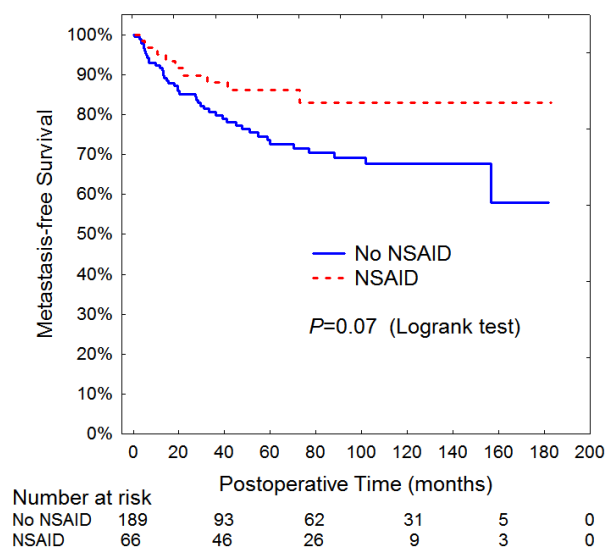


Figure 7. Kaplan-Meier curves of overall survival for 255 patients receiving or not intraoperatively a non-steroidal anti-inflammatory drug (NSAID), ketorolac or diclofenac during lung surgery for primary NSCLC. Univariate analysis by logrank test.

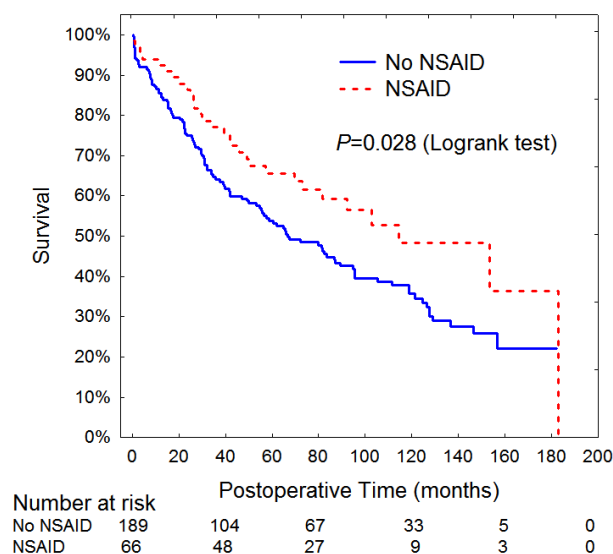


Figure 8. Kaplan-Meier curves of recurrence-free survival for 38 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) ≥ 4 , before breast cancer surgery in Centre 1 and receiving, or not, an intraoperative NSAID (ketorolac or diclofenac). Univariate analysis by logrank test.

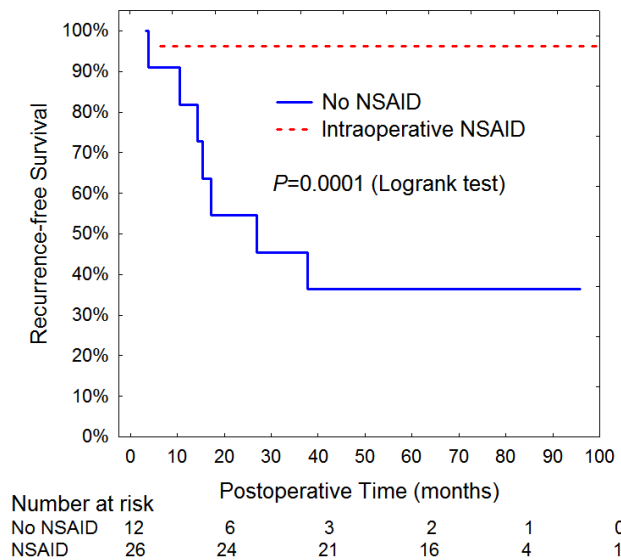


Figure A1. Kaplan-Meier curves of recurrence-free survival for 172 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) $<$ or ≥ 4 , before breast cancer surgery in Centre 1. Univariate analysis by logrank test.

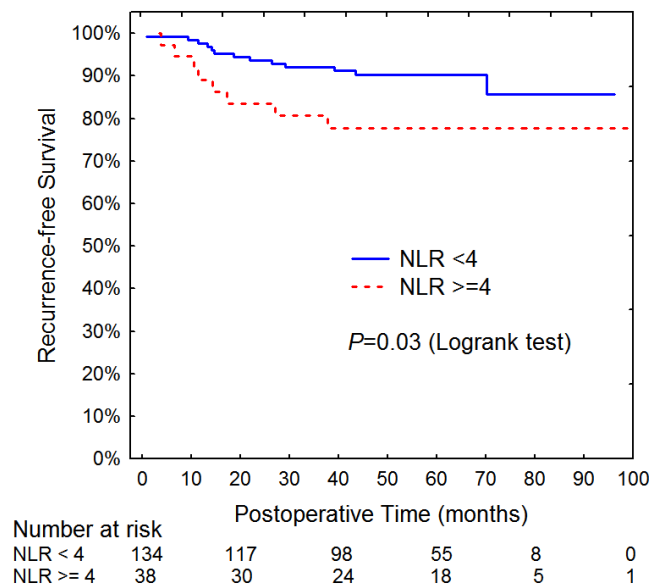


Figure A2. Kaplan-Meier curves of overall survival for 172 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) $<$ or ≥ 4 , before breast cancer surgery in Centre 1. Univariate analysis by logrank test.

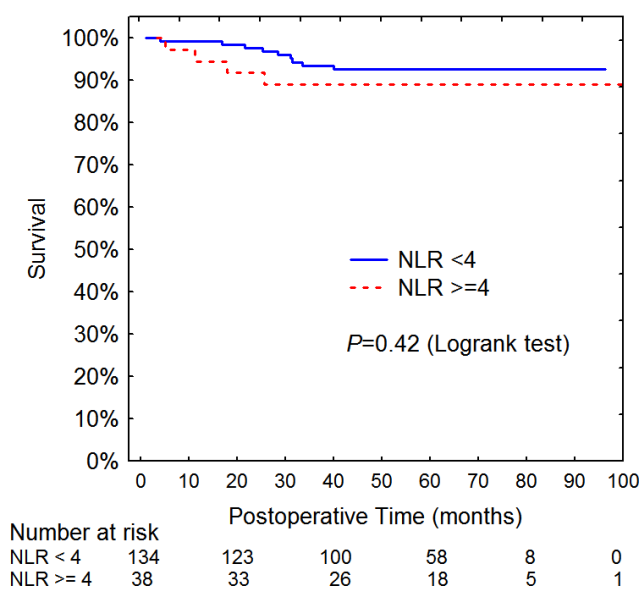


Figure A3. Kaplan-Meier curves of recurrence-free survival for 162 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) < or ≥ 3 , before breast cancer surgery in Centre 2. Univariate analysis by logrank test.

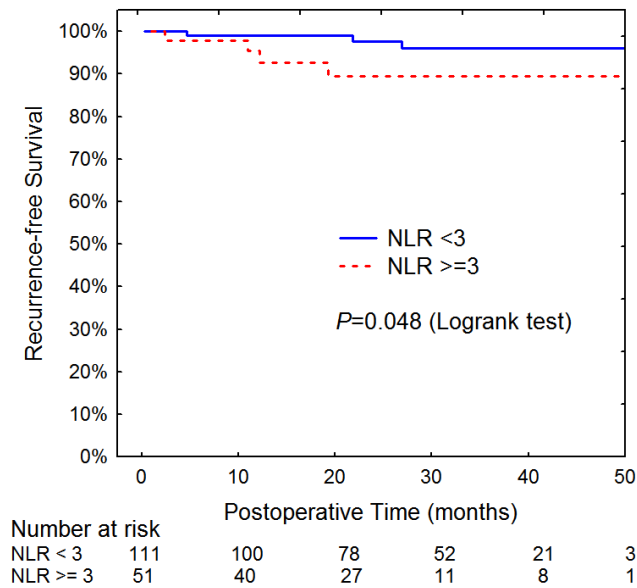


Figure A4. Kaplan-Meier curves of overall survival for 162 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) < or ≥ 3 , before breast cancer surgery in Centre 2. Univariate analysis by logrank test.

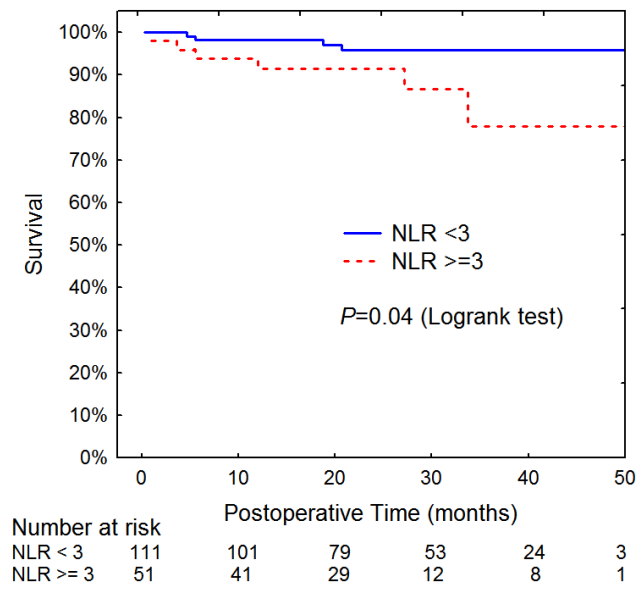


Figure A5. Kaplan-Meier curves of distant-metastasis-free survival for 255 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) $<$ or ≥ 5 , before lung surgery for primary NSCLC. Univariate analysis by logrank test.

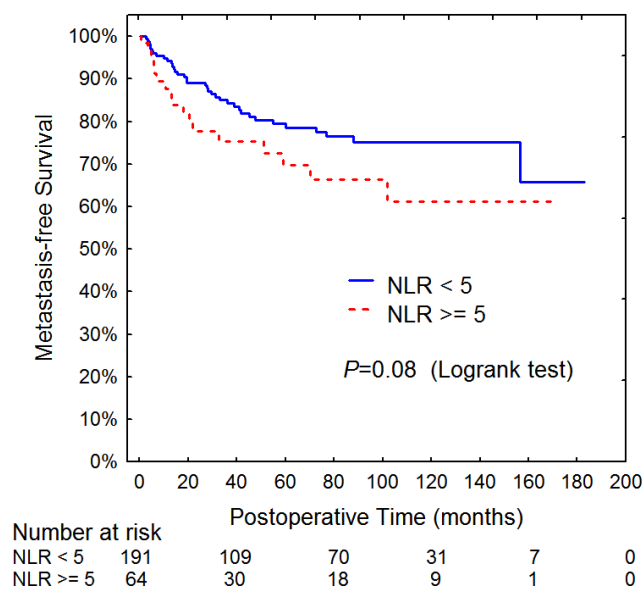


Figure A6. Kaplan-Meier curves of overall survival for 255 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) $<$ or ≥ 5 , before lung surgery for primary NSCLC. Univariate analysis by logrank test.

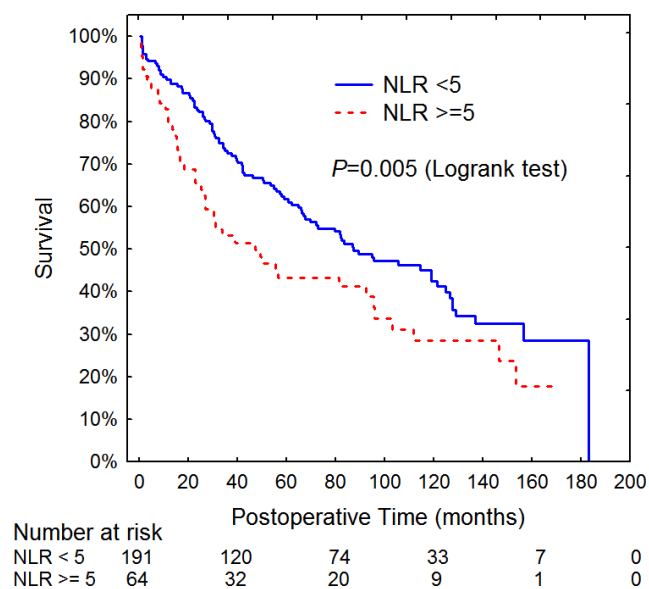


Figure A7. Kaplan-Meier curves of recurrence-free survival for 227 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) < or $\geq$ 5, before nephrectomy for kidney cancer. Univariate analysis by logrank test.

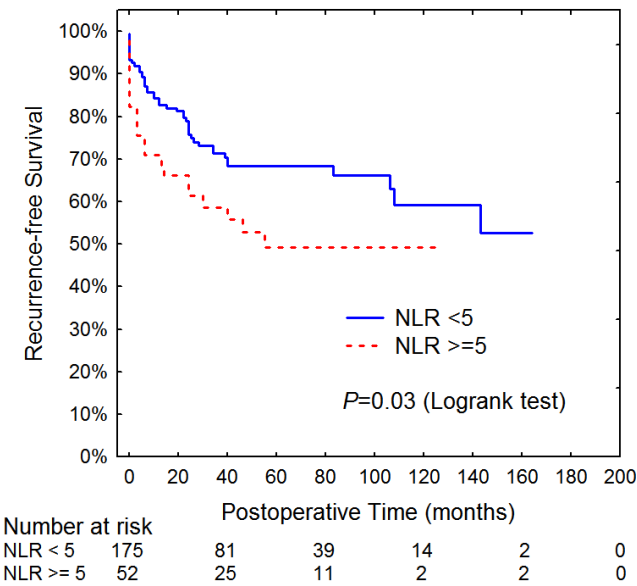
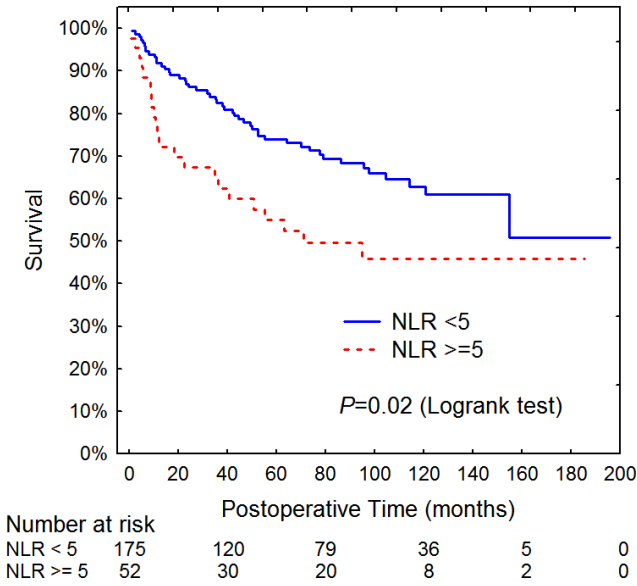


Figure A8. Kaplan-Meier curves of overall survival for 227 patients with a preoperative value of neutrophil:lymphocyte ratio (NLR) < or $\geq$ 5, before nephrectomy for kidney cancer. Univariate analysis by logrank test.



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SECTION 4

Conclusions and Perspectives

In this last text, we summarize the literature and the added value of our studies.

We describe how inflammation can be perioperatively monitored and proposed as a potential therapeutic target.

We highlight the added value of retrospective studies, unique opportunities to improve scientific knowledge.

We describe some ongoing trials and propose that biomarkers should be incorporated in clinical practice as well as in clinical research.

We give an example of a planned trial applying this kind of concept.

Finally, we conclude this series of studies as a coherent set permitting now to construct new models of clinical research in the perioperative period.

Perspectives in Anaesthesia for Cancer Surgery

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Conflict of Interest:

The authors attest no conflict of interest.

ABSTRACT

It is a fact that inflammation is an important prognostic factor in cancer surgery. Many data have been published last years showing that inflammation is a causative event in many cancers and a concomitant event in all malignant tumours. What is new is that we can assess inflammatory status during the preoperative period of our cancer patients with simple and widely available parameters, like the Neutrophil:Lymphocyte ratio. This kind of biomarkers will be helpful, for the clinicians, to stratify the patients and, for the researcher, to incorporate it in clinical trials. Promising clinical trials, focusing on perioperative inflammation, are ongoing. Rationale for these trials came from databases analyses. This kind of analyses must be extended to follow the long-term effects of our interventions. With this kind of work, we have shown a correlation between non-steroidal anti-inflammatory drugs, especially ketorolac, and improved outcome (metastasis-free survival and/or overall survival) in breast and lung cancer patients. Focusing on a high-risk group with preoperative inflammation could led to a clinical trial to test the effect of ketorolac on cancer outcome.

1. WHAT IS ALREADY KNOWN?

1.1. Inflammation is an important prognostic factor in cancer surgery

1.1.1. Implications of perioperative inflammation in cancer

Inflammation plays a central role in cancer. Before dissemination, inflammation can induce carcinogenesis, dedifferentiation and primary tumour growth (1) (Figure 1). After the dissemination step, inflammation promotes proliferation of tumour cells by inhibiting apoptosis and increasing mitosis rate (1). But inflammation contributes to the initiation of antitumor immune responses, mainly cellular-mediated immunity. Sensors of the innate immune system appear to be capable to detect tumours at an early stage, for example through soluble nucleic acids released from tumour cells, and trigger the local production of inflammatory cytokines that recruit immune cells such as lymphocytes, macrophages and dendritic cells. This janus-like role of inflammation is often referred to as the “paradox of inflammation” (2).

During and after cancer surgery, we and others have shown that systemic inflammation is an important prognostic factor (3,4,5,6,7,8,9,10,11,12). It was not actually a surprise since many data has been published last years showing that inflammation is a causative event in many cancers and a concomitant event in all malignant relapses (1).

But what is inflammation? Many different processes occur in cancer patients, especially with rapid tumoural growth, involving cytokines and other factors like Interleukin-1 β (IL-1 β),

Interleukin-6 (IL-6), Tumor Necrosis Factor (TNF- α), prostaglandin E2 (PGE2), Vascular Endothelial Growth Factor (VEGF) and Reactive Oxygen Species (ROS) influencing the number and the activity of immune cells like Natural Killer cells (NK), Tumour-Associated Macrophages (TAM), Tumour-Infiltrating Lymphocytes (TIL) and Myeloid-derived Suppressor cells (MDSC) (1).

To assess inflammation, the Neutrophil:Lymphocyte ratio (NLR) can be successfully used as a prognostic factor in surgical cancer patients (4-12).

1.1.2. NLR as a tool to screen and to monitor the patients

Many other tools than NLR have been described in the perioperative period. Cytokines, catecholamines, cortisol levels, leukocyte subtypes (granulocytes, monocytes, T lymphocytes subtypes, NK, NKT, CD4+CD25+ and B cells) and their surface markers (HLA-DR and LFA-1) have been proposed to screen and/or to monitor patients in the perioperative period (13,14,15). Bartal *et al* showed that several immune alterations are variably present prior to surgery (16). This fact highlights the need to use screening tools able to identify the differences existing between our patients. These differences contribute to the marked postoperative changes observed (16) but how these changes are linked to a different outcome is still debated (3). As a consequence, if we need for a biomarker, we should choose one that can recognize preoperative immune changes and that is clearly correlated to outcome. This allows a stratification of the patients' risk and, additionally, an identification of high-risk subgroups to test therapeutic interventions. Invariably, a high NLR value was shown to be deleterious in terms of tumour progression and was proposed as a tool for the clinician as well for the researcher (4-12).

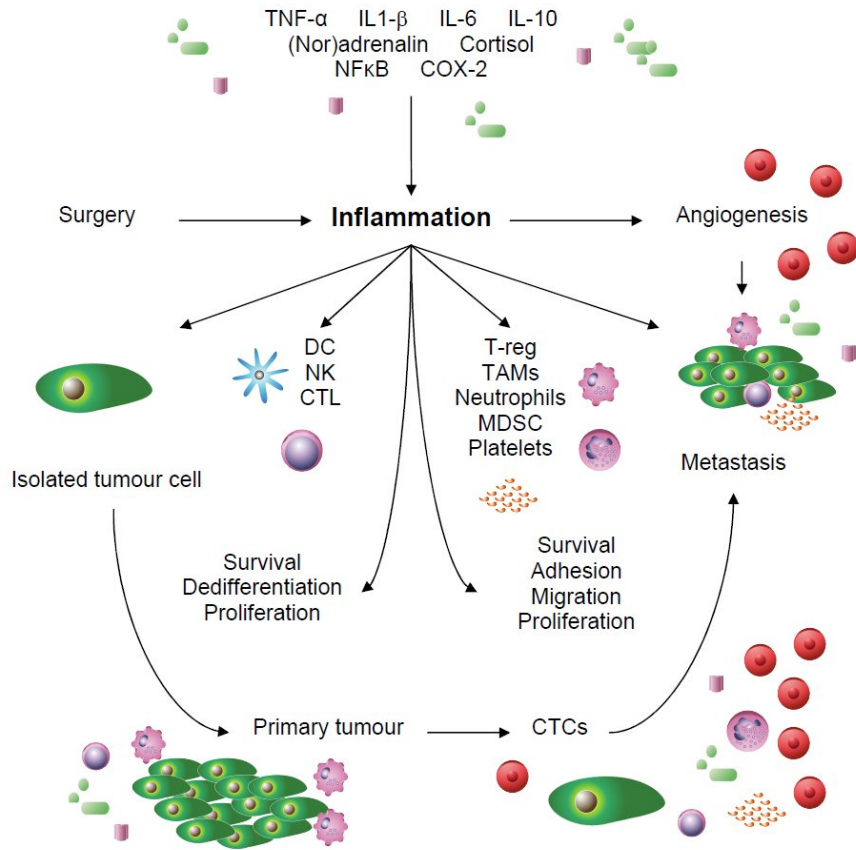


Figure 1. Surgery and inflammation are closely associated, and linked to mechanisms promoting tumour growth. At the time of the extirpation of the tumour, the incidence of circulating tumour cells (CTCs) depends of several mechanisms including inflammatory environment around the tumour itself. Inflammation promotes escape into the bloodstream, but also growth of metastases. Platelets can be involved in this dissemination process, by adhesion mechanisms and/or by the synthesis of mediators. Immune cells could both participate to the elimination of cancer cells (Natural Killer cells - NK, cytotoxic T lymphocytes - CTL, dendritic cells - DC) or to the suppression of the immune response (T regulator lymphocytes, T reg, tumour-associated macrophages and neutrophils, myeloid-derived suppressor cells - MDSC). COX-2 is overexpressed in tumour cells and in immune suppressor cells, like macrophages. Prostaglandins E2 (PGE2) could promote growth of the tumour directly and indirectly, via suppression of cellular-mediated immunity. The cytokines Interleukin-1 β (IL-1 β), Interleukin-6 (IL-6) and Tumor Necrosis Factor (TNF- α) can also suppress directly the activity of immune cells and promote the number and the activation of suppressor cells. Other factors, increased by surgery, accentuate this phenomenon, including (nor)adrenalin and cortisol levels. Anesthesia *per se* or by the impact against surgery-induced inflammatory process is able to interfere with many of these mechanisms.

1.2. Anti-inflammatory techniques are a promising way

Inflammation is linked to a pejorative outcome. We have shown that a single intraoperative administration of NSAIDs (ketorolac and diclofenac) could counteract this effect. Better outcome associated with NSAIDs use was, in breast cancer, a longer recurrence-free survival and in lung cancer, a better distant metastasis-free survival and overall survival (4,19). In breast cancer patients with a preoperative $\text{NLR} \geq 4$, the relative risk reduction of recurrence and mortality linked to the NSAIDs use is twice greater than in the whole series. This adds information not only for possible treatments, but also on cancer mechanisms. It highlights the role of systemic inflammation in the perioperative period of cancer surgery and how we could treat it. The mirror situation is also possible. Patients with tumours with a slow growth, typically with a low level of inflammation and a small risk of early relapse, could have a smaller benefit of NSAIDs. This observation was done in a large retrospective series of 1111 prostate cancer patients, operated from retropubic prostatectomy, without any difference in term of biological recurrence comparing patients receiving or not intraoperative ketorolac (17).

Taken together, these data show that anti-inflammatory techniques have a great potential in the perioperative period of cancer surgery, as proposed in non-surgical settings by the task force of the Society for Immunotherapy of Cancer, supported by the National Institute of Health (NIH). Our data suggest that the effect of NSAIDs, and potentially all other techniques with anti-inflammatory effects may depend of systemic inflammation level. Their influence should be analysed conjointly with inflammatory scores like the NLR. The lack of these conjoint analyses could explain discrepancies between studies focusing on the effects of locoregional analgesia or morphine in the perioperative period (18,19,20,21,22,23,24,25,26,27,28).

1.3. Why retrospective databases analyses are so important in research

1.3.1. Retrospective databases analyses helped to understand pathophysiology

From many years, oncologists proposed to revisit classical paradigms with database analyses (29,30,31). They showed that databases analyses can identify inconsistencies between theories and observations. An example is the discovery of bimodality in the relapse frequency over time in early stage breast cancer patients treated by surgery (31). An early peak of relapses, seemingly depended of the timing of surgery, is observed at 12-18 months. A broader second peak occurs at 60 months followed by a plateau lasting more than 15 years. We and others have done the same observation (20,32,33,34,35). As most relapses (50-80%) reside in the first peak, this is of major concern for the perioperative physician, thereby involved, at least

timely, in the pathophysiology of cancer relapse. Sometimes, this early peak appears only on smoothed hazard plots (35). Therefore, it appears that retrospective analyses are of prime importance to confront observations to theories and to identify potential therapeutic targets. This approach can be used to analyse the influence of our interventions and/or to improve comprehension of long-term outcome after anesthesia (36).

1.3.2. Retrospective databases analyses permitted to identify subgroups

Subgroups identification can be important to understand the effects of our interventions. It helped us in the comprehension of the effect of NSAIDs in breast and prostate cancer patients. Other examples exist, where development of drug candidates was stopped whereas it might have been successful. One condition of a successful test is to focus on patients most likely to benefit from the drug (36). The incorporation of biomarkers is, at least partially, a response to identify these patients. In our works, incorporation of the NLR led to a better understanding and documentation of the biology. It could prevent failure of clinical trials testing drug candidate (36). Another example is the high incidence of early relapse observed after breast cancer surgery in triple-negative histological type patients. A selection of patients based on this prognostic biomarker to test the influence of ketorolac has been proposed (32). This approach could reduce the high attrition rate and minimise futile exposure of patients to ineffective investigational therapies (36).

1.3.3. But retrospectives analyses are insufficient to definitively conclude: the example of NSAIDs

The use of retrospective analyses suffers from limits, precluding definitive conclusion. These limits can even, at least in some cases, lead to wrong interpretation. In the case of the NSAIDs effect detailed above, wrong interpretation may come from the following reasons: the statistical methods, an inclusion bias, an unrecognised confounder or a real effect but aspecific, anesthesiologist or analgesia-related. Concerning the statistics, a type I error remains possible, even if improbable seeing the repetition of the results. Overfitting, i.e. poor predictive performance or exaggerated minor fluctuations in the data, is also a well-known statistical problem of prognostic factor analyses; but testing in different databases and different centres has been proposed to control this risk (4). Uncontrolled and unrecognized biases are frequent in retrospective studies. In these cases, the use of prospective listing, high quality databases including all known variables that influence cancer outcome were analyzed. One problem could be the non-administration of the NSAID due to poor vital status (i.e. contra-indication to NSAIDs itself associated with a worse postoperative evolution). But most of the contra-indications, e.g. untreated active gastric ulceration or uncontrolled asthma, are

treated before surgery and consequently not anymore present intraoperatively. The only non curable contra-indication is severe renal insufficiency, but too rare in our patients to be able to impact significantly our results. Nevertheless, not able to know the exact prevalence, we cannot definitively exclude it. So, if not for contra-indications, why some patients did not received NSAIDs and other well? In our centres, the use of a single dose of NSAID was not standardized by an institution protocol. NSAIDs were administered depending from the preference of the anaesthesiologist in charge of the patient. Therefore, unrecognized bias in the patients or tumours characteristics is improbable. But a bias coming from the procedure is possible. Indeed, if it is probable that the patients receiving a NSAID, or not, had different anesthesiologists, it is possible that they were operated during a different day during the week (some anesthesiologists working a part of the week). The day of the surgery has been recently associated with a different outcome, the beginning of the week seeming better than the end (37). Moreover, the anesthesiologist in charge of the patient, or even the NSAID by itself, could have a non-specific effect on the outcome. We could speculate that communication skills of the anesthesiologist, efforts to provide a good postoperative analgesia and an earlier rehabilitation may be different. Effective NSAIDs-associated analgesia (but not specific to the mechanism) may also lead to a better postoperative evolution. Finally, we could speculate that the effect on the outcome may simply depends from the delay and the completion of postoperative adjuvant therapies (chemotherapy and/or radiotherapy) possibly depending from the anaesthesiologist and/or the used technique.

2. WHICH ARE THE PERSPECTIVES TO IMPROVE PATIENTS CARE?

2.1. What is the place of the perioperative Neutrophil:lymphocyte ratio?

An attractive role of high NLR to identify high-risk patients has been proposed in cancer and in non-cancer settings (4-12). It could be assessed in the preoperative period and followed after surgery. But why do not simply do it with other parameters like the C-reactive protein (CRP) as proposed in the modified-Glasgow prognostic score in cancer patients (12)? Before surgery, we already know that those parameters do not necessarily reflect the same phenomenon, since these depends from, at least in part, different mechanisms (16). After surgery, these differences are evident as evolution of NLR and CRP are not parallel (38,39). An analysis of a cohort of patients undergoing major abdominal surgery showed that correlation between NLR and CRP was moderate before surgery and at postoperative day+7 ($R^2=0.40$ and 0.38 , $P<0.05$). In contrast, even if statistically significant, correlation was poor at day+1 and +2 ($R^2=0.06$ and 0.10 , $P<0.05$) (37). Interestingly, at day+7, an increased NLR

was the only independent parameter associated with more complications ($P < 0.001$). This confirms previous observation of Vaughan-Shaw *et al* and Cook *et al* (39,40). The importance of a persisting high NLR value after one week has been associated not only with short term morbidity (cardiovascular and septic) but also with higher long term mortality after lung cancer surgery and orthopaedic surgery for hip fracture (4,41).

Taken together, we propose that the NLR would be used to screen and to monitor patients' response to cancer and to surgery. This monitoring could be used in clinical practice from diagnosis to the end of the postoperative recovery. This could also be used as an inclusion criterion in clinical trials.

2.2. Ongoing trials to modulate inflammatory pathways should incorporate biomarkers

2.2.1. Examples of ongoing trials

NSAIDs or aspirin are proposed in general population suffering from curable cancer undergoing surgery. In most of these studies, selection of patients does not include a biomarker. Non-exhaustively, we give here some examples. Preoperative aspirin to improve postoperative survival is now studied in lung cancer surgery (NCT01058902). It could influence overall survival either by cancer- and non-cancer-related mortality. Focusing specifically on cancer-related endpoints, etodolac is proposed to prevent recurrence of colorectal tumours, in combination with a β -adrenergic blocker (Propranolol) (NCT00888797). To focus on a more determinant event, some investigators propose correlative studies, i.e. testing the influence of a NSAID on one or several biomarker. For examples, indomethacin or celecoxib are tested in colorectal and breast cancer surgery with tumour immunity and gene expression as endpoints (NCT00473980 and NCT01695226). Celecoxib is tested to suppress aromatase activity in breast cancer patients (NCT00070057). Ibuprofen is tested with concentration of the cytokines TNF-alpha, IL-1Beta, IL-2, IL-6, IL-10, and IFN-gamma and prostaglandin E2 as primary outcome (NCT01377441).

In non-cancer studies, investigators study NSAIDs or aspirin for cardiovascular prevention. One of the greatest trial conducted now focus on the effect of aspirin in the perioperative period to prevent cardiovascular morbidity/mortality in the PeriOperative Ischemic Evaluation-2 Trial (POISE-2) (NCT01082874). This is an example of large database (10,000 patients) that should be used to monitor the effect of aspirin on cancer recurrence, even if not designed for this goal. Unfortunately, we cannot exclude important missing information, leading to inconclusive results as we depicted above in analyses of analgesic techniques on cancer outcome. All these studies (outcome studies and correlative studies, i.e. with

biomarkers as endpoints) are interesting. But we propose now to perform trials on biology selected subgroups of patients.

2.2.2. An example of the incorporation of a biomarker in a cancer trial

We have described above the promising place of NSAIDs, like ketorolac, in cancer surgery. We have also described why it is logical to incorporate the NLR as an inclusion criterion to test the effect of perioperative ketorolac on recurrence-free survival in breast cancer patients. If we could confirm the reduction of early recurrences rate observed in retrospective studies, the implications for patients and for public health will be major; taking into account that ketorolac is a non-toxic, inexpensive and widely used analgesic (4,20). The incorporation of the NLR as an inclusion criterion would be a typical application of the new model of drug trial recently proposed by Lacombe *et al.* By a pragmatic approach we confirmed the importance of the selection of high risk subgroups. We retrospectively tested the influence of ketorolac in a centre with a low recurrence rate after breast cancer surgery. The ketorolac group presented more than twice less recurrences than patient without ketorolac. But the low number of events concomitantly with a low recurrence rate (5.6% in the whole series) limited the power of the analysis and the reduction of relapse risk did not reach statistical significance. To have a statistically significant effect, we should have a largely greater number of patients (thousands of patients, more than we could find in most centres). A second reason is to test the impact of ketorolac in a population where the biology is related with the target of ketorolac. It is difficult to speculate on the possible underlying mechanisms modulated by ketorolac but, as facts, we can observe what happened in our patients. Analysing the time frame of recurrences in our series of breast cancer patients, we seen that the reduction of recurrences risk is clear for the earlier recurrences (occurring months after surgery) and not so clear for the later (occurring years after surgery) (32). As a consequence, it could be very interesting to investigate the effect of ketorolac in patients with the highest risk of early recurrence, most likely to benefit from the drug. For this goal, we propose to test the effect of ketorolac specifically in patient with a high NLR.

3. CONCLUSIONS

Inflammation is a major prognostic factor in cancer surgery as well as in nonsurgical settings. Biomarkers like the NLR can reflect this process and can be now used in clinical practice and integrated in clinical research. Biomarkers are now proposed not only in cancer surgery, but also to identify high risk subgroups in non cancer surgery to potentially prevent morbidity and mortality, or at least to treat preventable risk factors. We have identified a promising role of

NSAIDs in the perioperative period of cancer surgery. We describe how to incorporate the NLR in a prospective trial testing the influence of ketorolac to prevent early relapses after breast cancer surgery. This illustrates that databases analyses and incorporation of biomarkers in clinical research will probably take a great part in the future.

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