Seasonal patterns of presentation in primary malignant brain tumors and metastases based on a retrospective neuropathologic database

Running Title:

Seasonality of adult brain tumor presentation

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ABSTRACT

Seasonal variation in the occurrence of several classes of cancer has been observed in the past. However, evidence for such trends in adult central nervous system tumors is scant. We have analyzed the monthly occurrence rates of glioblastomas as well as carcinomas metastatic to the brain in 6,154 neurosurgical patients in Toronto selected from the University Health Network neuropathologic database over a seven-year period (July 2001 to June 2008). The electronic repository was representative of the patient population in southern Ontario, and the case accession dates in the database reflected the onset patterns of the selected tumor groups. A modification to Nam's alternative method to the Roger test was developed to statistically quantify the differences. The results demonstrated significant cyclical occurrence rates of glioblastomas with seasonal peaks in March, June, September and December. Moreover, significant increases in the rates of carcinomas metastatic to the brain were found for January, April and August. Surprisingly, the monthly frequency for the two tumor groups resembled each other in peak/trough topology. Semiquantitative comparison of major histologic features between glioblastomas from a peak (March) and trough (November) month in the seven-year period was performed, revealing differences in the amount of perivascular lymphocytic inflammation. This novel observation may have profound implications for the understanding of the biology of adult central nervous system tumors.

KEYWORDS

brain tumors; epidemiology; glioblastoma; inflammation; metastatic carcinoma; seasonality

INTRODUCTION

Understanding seasonal variations in disease occurrence can provide insights into pathogenesis which may have diagnostic and therapeutic implications. The identification of seasonal trends for a number of cancers has led to the investigation of potential causative environmental factors. Examples include prostate [1], thyroid [2] and skin cancers [3], which have been linked to the effect of varying daylight and UV radiation on vitamin D synthesis. Exposure to infectious agents has been implicated in the seasonality of cervical cancer [4], lymphomas [5] and frog adenocarcinomas [6, 7]. Similar correlations have been proposed for breast cancer with pineal biorhythms and expression of progesterone receptor [8]. However, no definitive consensus for seasonal patterns in breast cancer exists [9, 10].

Associations between season of birth and occurrence of brain tumors have been reported [11, 12], pointing to possible pre- or postnatal effects. To our knowledge, the only reported observation of seasonal variation in adult malignant brain tumors was made from the Tumorzentrum Regensburg database in southeastern Germany [13]. The investigators reported circannual periodicity for glioblastomas in women but not in men.

The current study presents further evidence for periodicity in the occurrence of glioblastomas and carcinomas metastatic to the central nervous system (CNS) over a seven-year period in Toronto, Canada. In addition, histological analysis of glioblastomas suggests the presence of seasonally-variable inflammatory and vascular events.

PATIENTS AND METHODS

Cases were selected from a neuropathologic database created from print and electronic archives at Toronto's University Health Network spanning 1971 to the present and

containing more than 31,000 specimens [14]. A seven-year period was chosen for the current study (July 2001 to June 2008). Two groups of tumors were selected for review: glioblastomas and metastatic carcinomas. Recurrent or previously treated cases were excluded. Anatomic locations were restricted to the cerebrum and cerebellum. Tumors were grouped by month of surgery, and frequency was calculated relative to the total number of neurosurgical cases in that month. Gender-based trends were also assessed. All queries of the database conformed to the University Health Network Research Ethics Board guidelines. In particular, no information was extracted from the repository that could lead to the recognition of patient identities.

To investigate the biological basis of seasonal trends in glioblastomas, histopathology was compared between a peak (March) and a trough (November) month of frequency. Hematoxylin and eosin-stained sections of these tumors were reviewed by two neuropathologists (S.E.C. and T.R.K.) blinded to the month of surgery. Six major microscopic features (necrosis, pseudopalisades, microvascular proliferation, thrombosis, perivascular lymphocytic inflammation and hemosiderin deposition) were scored semiquantitatively as absent (0), minimal (1) or extensive (2).

Statistical analyses for seasonality studies are classically performed using the Roger test [15]. An alternative method increases power by utilizing the premises of the hypothesis being examined [16]. It assumes two seasons to be proportionally constant, the third to have peaked, and the fourth to be below average. For the seasonality analyses in this study, we used a modification of Nam's method (see **Supplement**). The major peaks in each trend were tested for significance against other monthly values, which were assumed to have minor variation relative to the yearly average. Statistical significance tests for histological

analyses were performed using a two-sample, one-tailed Student's *t*-test assuming equal variance, with *P*-values less than 0.05 considered significant.

RESULTS

Among the 6,154 neurosurgical cases in the selected period, 505 glioblastomas (316 males, 189 females, mean age = 58.6 ± 14.3) and 386 metastatic carcinomas (167 males, 219 females, mean age = 59.6 ± 11.8) were found. Gender distribution among all cases was 48.3% female and 51.7% male, with no particular gender being overrepresented in a given month. The frequency trend for glioblastomas showed highly significant peaks in March, June, September and December (Z = 3.329, P = 0.0004), and metastatic carcinomas demonstrated peaks in January, April and August (Z = 2.697, P = 0.0035) (**Figure 1A**). Therefore, the peak/trough patterns of the two tumor groups closely resembled each other. Furthermore, no significant gender differences were observed (**Figures 1B, C**).

Histologic evaluation of all glioblastoma cases from a peak (n = 33) and trough (n = 32) month demonstrated a significant increase in the amount of perivascular lymphocytic inflammation (P = 0.048) in the trough month (**Figure 2**). There were no significant differences in other histologic features, namely thrombosis (P = 0.167), hemosiderin (P = 0.175), pseudopalisades (P = 0.208), necrosis (P = 0.233) and vascular proliferation (P = 0.336).

DISCUSSION

We have demonstrated significant seasonal variation in the presentation of both glioblastoma and CNS-metastatic carcinoma. The degree of malignancy and rapid growth

of these lesions usually mandates surgery within a short time after first clinical symptoms, ensuring a close correlation between initial presentation and the true occurrence of the disease. Moreover, the use of the frequency of diagnosis as a fraction of total neurosurgical cases rather than the number of diagnoses in this study eliminated potential effects of external variables, such as the number of neurosurgeons on duty or level of experience of the surgical staff in any given month. In addition, the months chosen for histological analysis did not constitute typical vacation times or periods of unusual surgical load.

The trend found for glioblastoma cases in our series is similar to that reported by Koch and colleagues (2005), who demonstrated significant peaks in March and September in female patients. Unlike that study, however, no gender differences were found in our series. The number of glioblastomas analyzed in the mentioned report (501 cases between 1992 and 2003) was comparable to the study presented here. The male : female ratios (approximately 1.5 : 1) for glioblastomas were also consistent with reported values [17].

Alternative statistical methods for seasonality analyses based on the date of clinical diagnosis and utilizing the von Mises distribution and angular regression methods have been suggested [18]. However, due to the existence of technical difficulties with these approaches as detailed by Gao *et al.* (2006), and the configuration of our database, we decided to group our data on a monthly basis and develop an extension to a model suggested earlier (see **Supplement**).

Histological comparison of glioblastomas revealed an increase in perivascular inflammation in a trough month (**Figure 2**). This observation could (i) suggest a protective role of inflammation, or (ii) point to an anticipation for an increased number of glioblastoma cases following the trough month. Seasonality in CNS vascular events is a

well-established phenomenon, as demonstrated by variation in the onset of primary intracerebral haemorrhage [19], spontaneous carotid artery dissections and intracranial aneurysms [20]. Seasonal variation in cytokine response has also been documented. Levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) after endotoxin stimulation result in a pro-inflammatory reaction in early summer and an anti-inflammatory reaction in early autumn [21]. This variation could theoretically manifest itself as the change in perivascular inflammatory infiltrate observed in this study. This cytokine pair may also play a role in the progression of gliomas [22, 23]. Moreover, it is possible that the development of malignant carcinomas to a metastatic stage is related to underlying circannually-varying factors, as infiltrative edges in breast cancer have been observed more frequently in the first half of the year [24].

Definite conclusions about associations between the observed trends in malignant brain tumors with environmental variability in the Northern Hemisphere are still premature. The effect of temperature on metastasis of renal carcinoma in frogs, which has viral origins, has been described [25]. Furthermore, release of interleukins and other cytokines by rhinoviruses or in influenza infections has been established [26, 27]. A possible link between seasonal temperatures, common infection rates in the general population and cancer metastasis warrants further investigation. Additionally, circannual changes in vitamin D levels, small-molecule hormones (e.g. melatonin), glucose intake and atmospheric aerosol levels together with seasonal changes in the protective actions of activated glial cells and inflammation could be future areas of research. As a direct follow-up to our study, histological analysis could be expanded to include additional years and immunohistochemical studies for cytokines and their receptors, hormone receptors and

other markers. The findings could also be correlated with neuroimaging, treatment and outcome.

In summary, we present evidence for seasonal variation in the presentation of malignant primary and metastatic brain tumors. We suggest that further studies linking this to seasonal variations in pro-inflammatory cytokines may help to understand biological events underlying the progression of these neoplasms. As the population in southern Ontario, and consequently their representation in our database, is very heterogeneous (2006 Census), we expect that our findings are not limited to one genetic group and could prove true in other geographic regions. This could lead to diagnostic and therapeutic conclusions with widespread applicability.

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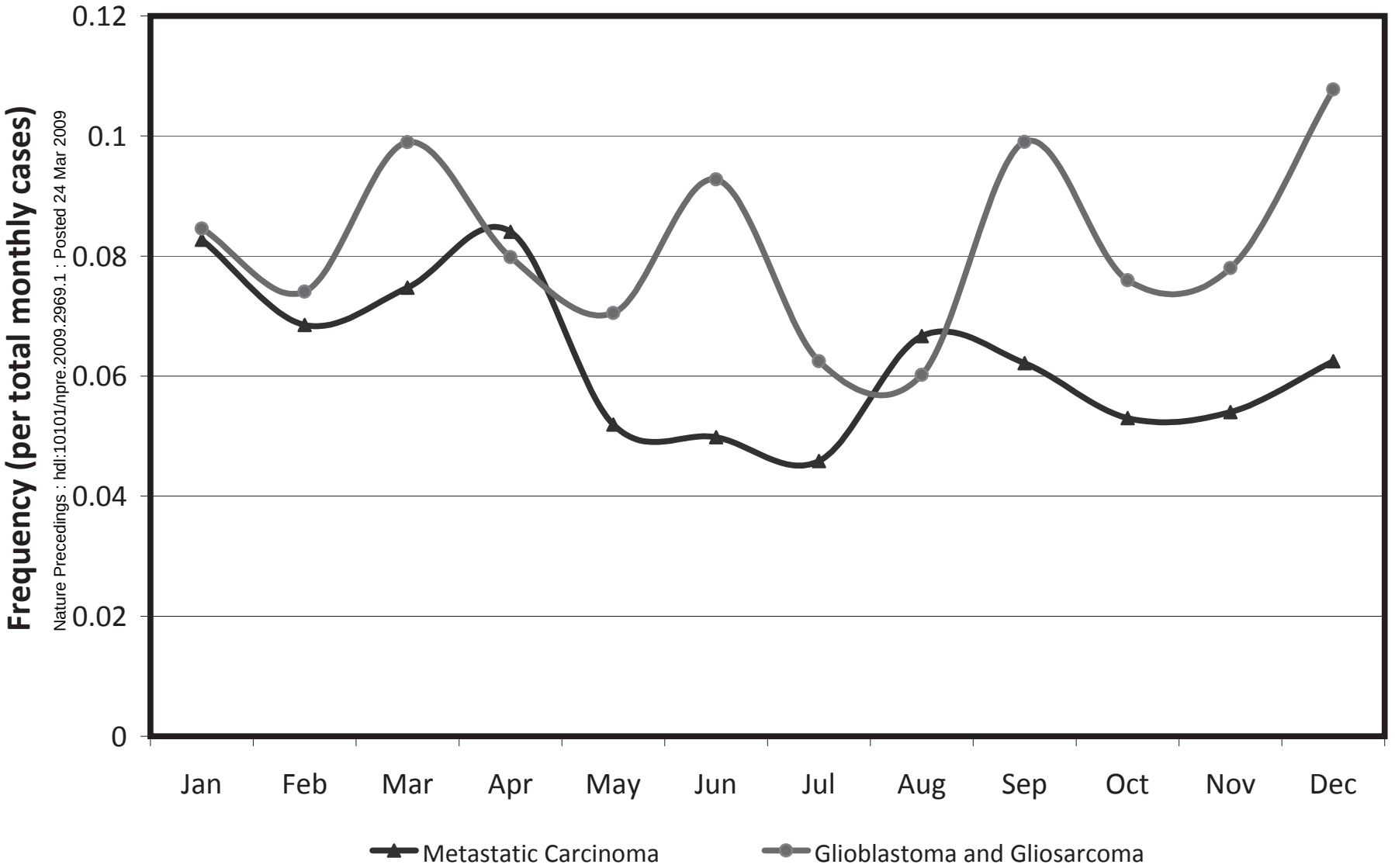
FIGURE LEGENDS

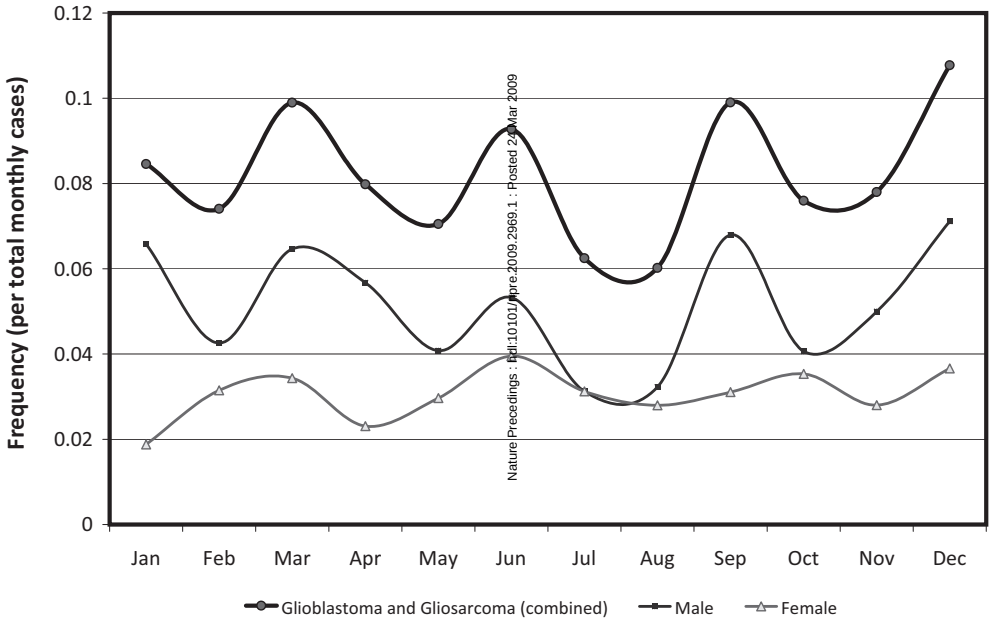
Fig. 1: Seasonal patterns of presentation. (A) Analysis of frequency of glioblastoma cases per total monthly cases revealed highly significant peaks in March, June, September and December. For metastatic carcinomas, there were peaks in January, April and August. Frequencies of glioblastoma diagnoses therefore closely resemble the trend for metastatic carcinomas. Gender-specific analysis demonstrates close resemblance of the combined graphs, notwithstanding male bias in glioblastoma cases (B) and female bias in the combined metastatic carcinoma cases (C).

Fig. 2: Histological comparison. Glioblastoma cases from a peak and trough month were compared in six major neuropathological features. The trough month showed a significant increase in the amount of perivascular lymphocytic inflammation.

Figure 1A.



1B.



1C.

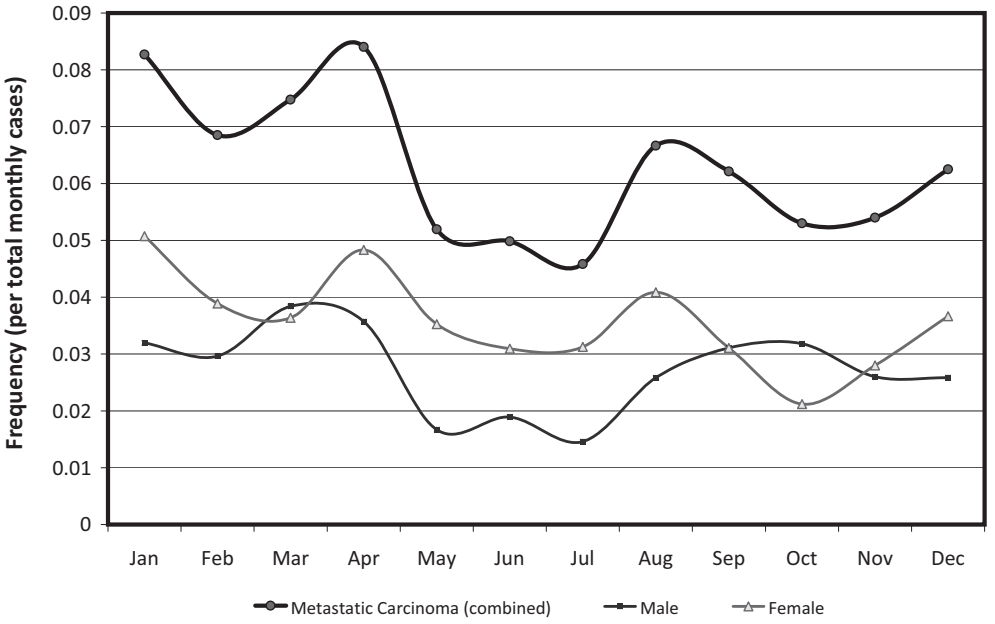
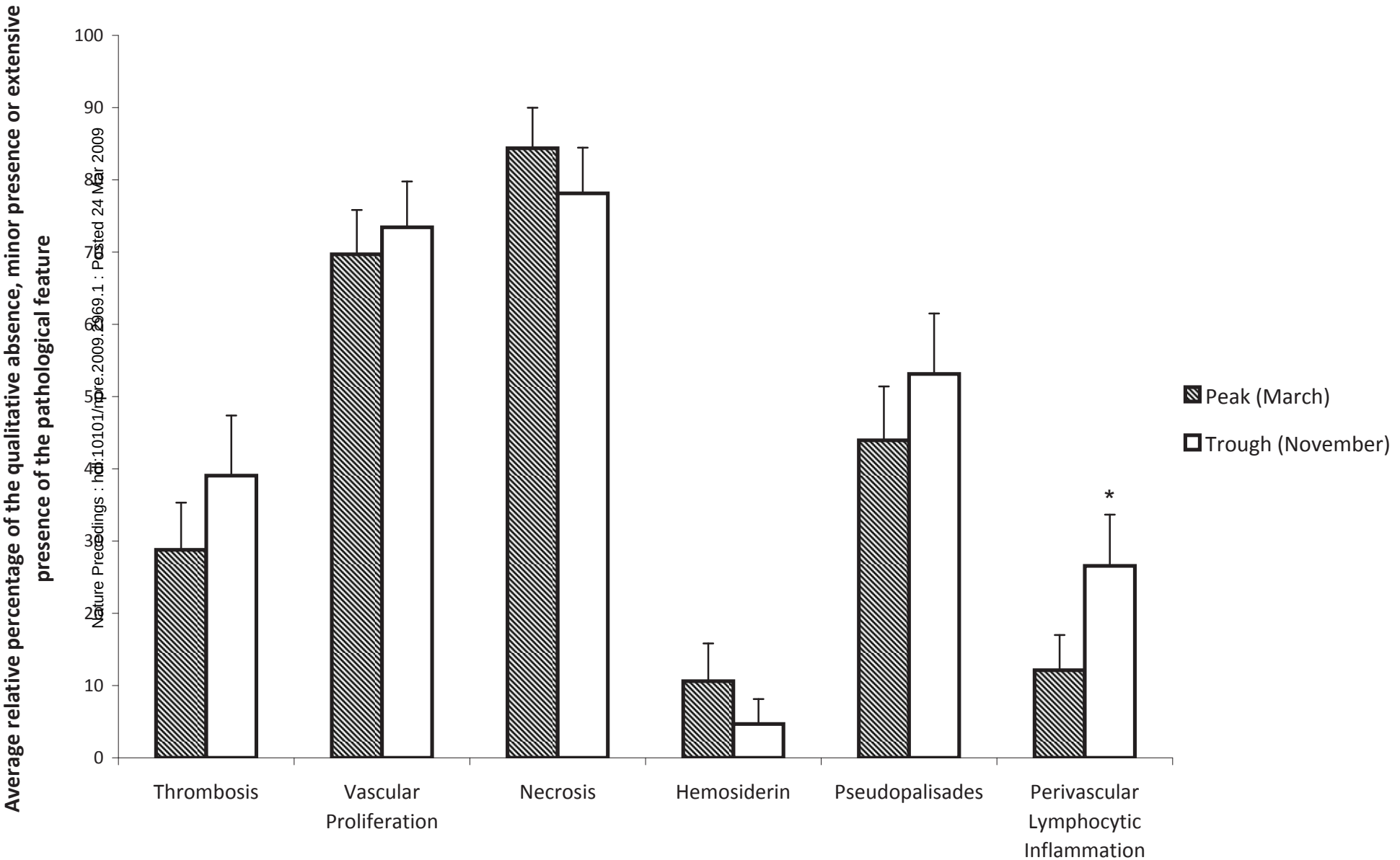


Figure 2.



Modified Score Statistic

We assume we have the following data:

$t_1, t_2, t_3, \ldots, t_{12} \rightarrow$ counts of specified tumour in months 1 through 12

$n_1, n_2, n_3, \ldots, n_{12} \rightarrow$ total tumour counts in months 1 through 12

Also define $p_1, p_2, p_3, \ldots, p_{12}$ be the proportions of the specified tumour in months 1 through 12. Note that we do not necessarily have $p_1 + p_2 + p_3 + \ldots + p_{12} = 1$; however, if there is no seasonality then $p_1 = p_2 = p_3 = \ldots = p_{12}$.

We suspect that p_1 and p_2 (say) may be higher than the other p_i's. This can be modeled as follows:

$$p_1 = p_2 = p + b \qquad p_3 = \ldots = p_{12} = p$$

where p and b are unknown parameters. For this model, we want to test the null hypothesis

$$H_0 : b = 0$$

against some appropriate alternative hypothesis H_1
(either (i) $b \neq 0$, (ii) $b > 0$, or (iii) $b < 0$).

Using a modification of the multinomial model in Nam (1995), it is possible to derive a score test for H_0. The sign test statistic for testing H_0 can be computed as

$$Z = \frac{N}{D}$$

where

$$N = (t_1 + t_2)(n_3 + \ldots + n_{12}) - (t_3 + \ldots + t_{12})(n_1 + n_2)$$
$$D = \{(t_1 + \ldots + t_{12})(n_1 + n_2)(n_3 + \ldots + n_{12})\}^{1/2}.$$

The rejection region for an α level test of H_0 depends on the alternative hypothesis:

$H_1 : b \neq 0$ reject H_0 for $|Z| > z_{(1-\alpha/2)}$

$H_1 : b > 0$ reject H_0 for $Z > z_{(1-\alpha)}$

$H_1 : b < 0$ reject H_0 for $Z < -z_{(1-\alpha)}$

where $z_{(1-\alpha)}$ and $z_{(1-\alpha/2)}$ are as defined in Nam (1995).