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Histological heterogeneity of human glioblastomas investigated with an unsupervised neural network (SOM)

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Summary. The histological variability of Glioblastomas (GB) precludes the modern assimilation of theses tumors into a single histological tumor group. As an alternative to statistical histological evaluation, we investigated 1489 human GB in order to discover whether they could be correctly classified using Self-Organizing Maps (SOM). In all tumors 50 histological features, as well as the age and sex of the patients, were examined. Four clusters of GB with a significance of 52 (maximal significance 60) were found. Cluster C1 contained 37.47% of all GB and 41.09% of all polymorphic glioblastomas (PG). Cluster C2 included 35.06% of all GB and 44.96% of all giant cell glioblastomas (GCG). Cluster C3 contained 16.45% of all GB with a significant component of astroblasts, glioblasts and oligodendroglia. Cluster C4 included 11.01% of all GB, 87.80% of the gliosarcomas (GS) and 36.72% of all GCG. Placing a series of component windows with their maps side by side allows the immediate recognition of the dependencies on variables and the determination of variables necessary to build the specific clusters. The SOM allow a realistic histological classification, comparable to the actual classification by the WHO. In addition, we found new, small subclusters of human GB which may have a clinical significance. With SOM one can learn to discriminate, discard and delete data, select histological and clinical or genetic variables that are meaningful, and consequently influence the result of patient management.

Key words: Neural networks, SOM, Glioblastoma, Clustering and classification

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Introduction

The histological type and grade heterogeneity of gliomas and GB is well known. However, quantitative systematic morphological data are not available. The glioblastoma was defined by Virchow (1863) as a tumor of glial origin. In 1926, Baley and Cushing (1926) used the name 'Glioblastoma multiforme' for the first time. Río Hortega (1932) and Polak (1966) described the 'isomorphic glioblastoma' as a tumor constituted of little round glial cells which is distinct from a glioblastoma multiforme. As the term 'multiforme or polymorphic' implies, the most salient feature is the diversity of tissue patterns and cell forms encountered: anaplastic astrocytes; gemistocytic, fibrillary or pilocytic areas; and the presence of astroblastic, oligodendroglial, and dedifferentiated cells. A systematic description of some qualitative histological features in GB was published by Zülch (1986). The histological variability from area to area and between different GB within the group as a whole precludes the modern assimilation of theses tumors into a single histological picture (McLendon et al., 1998). There is evidence that the variability of GB is genetically determined (Hayashi et al., 1997; Kleihues and Ohgaki 1997; Meyer-Puttlitz et al., 1997; Duerr et al., 1998) Phenotypic histological, immunohistological and biological heterogeneity is essentially an expression of genotypic variability (Ironside et al., 2002). The histological features form the basis of prognostic inferences and of therapeutic decisions, therefore they are of paramount importance in evaluating the effectiveness of therapeutic strategies. Age, tumor necroses, diffuse infiltration of tumor cells, number of mitoses and other histological features may determine the postoperative survival and recurrences (Budka et al., 1979; Burger and Vollmer, 1980; Schmidt, 1983; Giangaspero and Burger, 1983; Burger et al., 1985; Nelson et al., 1985; Burger and Green, 1987). Significance of individual proliferation rates is still under discussion (Hopf et al., 1994). Each single factor may be statistically significant to determine the survival

of patients with GB. However, the biological evolution of the tumoral process could be conditioned by all variables. As an alternative to statistical evaluation of histological features we investigated, in a large series of patients, whether human GB can be grouped in different clusters on the basis of their histological heterogeneity using Self-Organizing Maps (SOM), an unsupervised neural network learning technique (Bock, 1997; Kohonen, 1995, 1997).

Material and methods

Data set

1489 human GB were selected and recorded between June 1976 and December 2003. Anaplastic astrocytomas, anaplastic oligodendrogliomas and astroblastomas were not included in the present series. All cases underwent a total or subtotal surgical excision. In every case all extirpated material was available for histological study. It was fixed in 10% formaldehyde and embedded in Paraffin. From 1976 until 1981, ten-µm sections of all tumors were dyed with hematoxylin and eosin, trichrome stains (Goldner, Mallory or Elastic van Gieson) and silver impregnations for astrocytes and oligodendroglial cells after Rio Hortega. Since 1982, each tumor was also assessed with polyclonal antiserum against Glial fibrillary acidic protein (GFAP), Vimentin (for the morphology of glial cells) and KI67 (Dako, Denmark), and with the acid phosphate anti-acid phophatase technique (APAAP).

In all tumors, 50 histological characteristics were examined and each one was graded on a scale of four: (zero: absence of the feature, one: present to a small degree, two: moderate presence and three: abundant). A detailed description of these 50 histological features has been given in previous studies (Cruz-Sánchez et al., 1988; Iglesias-Rozas, 1986; Iglesias et al., 1983, 1985, 1986, 1988). The histological features together with the age and sex of the patients were used as the basis of the classification.

SOM technique

Viscovery SOMine Standard Edition Version 3.0 (Eudaptics software GmbH, Hauptstrasse 99, A-4232 Hagenberg, Austria) is a tool based on the concept of Self-Organizing Maps (SOM). This is a powerful tool for analyzing and visualizing complex data sets without prior statistical knowledge. In Viscovery SOMine, the SOM are done by using a two-dimensional hexagonal grid (a map) with 47x43= 2021 nodes or neurons. Initially, the histological data of each glioblastoma are associated to a random node in the grid. The software performs a sequence of steps that bring nodes with similar data close to each other. At the end of this process, which takes about 5 minutes, the grid has been split into regions corresponding to the mathematically most significant classification of the glioblastomas into subgroups. The software allows to visualize different aspects of the classification, evaluate similarities between components, monitor new data, and retrieve cluster statistics. Placing a series of component windows side by side allows the immediate visual and statistical investigation of the dependencies on variables. Each node in the neighborhood will be colored: The deeper the color the stronger the similarity.

Results

In our series of 1489 cases, 872 (58.56%) patients were male and 617 (41.44%) female. The youngest patient was 1 year old, the oldest 93 years old with a mean of 55 years. Based on the WHO classification (Kleihues and Cavenee, 2000). 1319 (88.58%) cases were diagnosed as polymorphic glioblastoma (PG), 129 (8.66%) cases as giant cell glioblastoma (GCG) and 41 (2.75%) cases as gliosarcomas (GS). Viscovery SOMine displays the Cluster window for GB with four clusters and a significance of 52 (minimal = 0, maximal value 60) (Fig. 1).

Each hexagonal unit, called node, represents a glioblastoma with its characteristic feature. The Cluster window displays colored map regions constituted by these clusters (Fig. 2). Clusters contain different numbers of GB, between 164 and 558 cases (Table 1).

In the lower right cluster (C1) 558 cases of GB, 542 PG, 15 GCG and 1 GS were included (Table 2 and Fig. 2). The top left cluster (C2) included 522 tumours, 461PG, 58 cases of GCG and 3 GS. The upper right cluster (C3), included 245 of GB The lower left cluster (C4) included 164 (11.01%) GB and 36 cases of GS (87.8% of all GS). Most GCG were included in the C2 cluster (44.96%). Fig. 2 The cluster window displays a colored map of the four clusters of human GB. Placing the series of component windows side by side allows

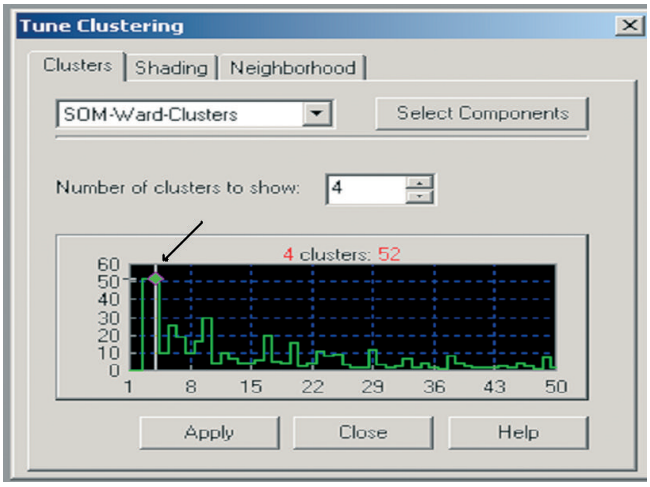

Fig. 1. Graph showing the four clusters which are possible and their corresponding significance of 52 (maximal value 60).

the investigation of the dependencies on features (Fig. 3).

The second important clustering included 10 clusters with a significance of 29 (Fig. 1). In this case (Fig. 4) *cluster C1* contained a subcluster (on the right) that included cases with scanty anomalous vessels (ANOMA), slight thromboses (THROM), little polymorphic cells (ZPOLY), small cells (G-ZELL), and a small quantity of polymorphic nuclei (KPLY).

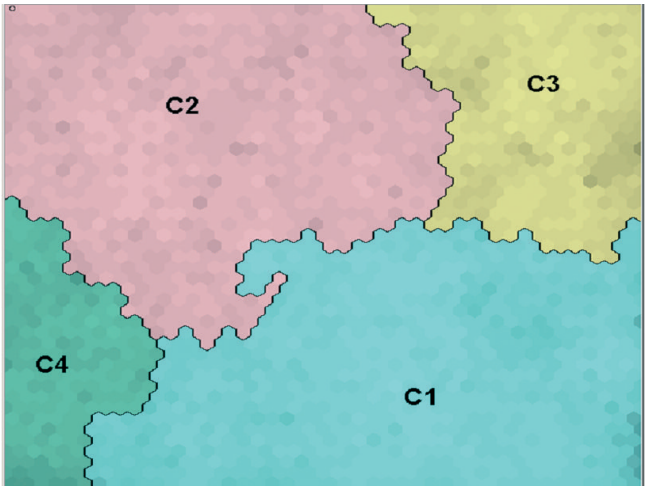

Fig. 2. The cluster window displays a colored map of the 4 clusters of human glioblastomas. A cluster may be seen as a map area containing similar vectors of similar histological cases of GB delimited by separators. A separator is the line that is drawn between two nodes if they are further apart than the cluster threshold. A component window represents the (average) component value at each node with a certain color. The scale below the picture shows the correspondence between colors and component values.

Table 1. Distribution of glioblastomas in the four clusters.

CLUSTER	PG		CGC		GS		TOTAL	
	Nr	%	Nr.	%	Nr.	%	Nr.	%
C1	542	41.09	15	11.63	1	2.44	558	37.47
C2	461	34.95	58	44.96	3	7.32	522	35.06
C3	235	17.81	9	06.98	1	2.44	245	16.45
C4	81	6.41	47	36.72	36	87.80	164	11.01
TOTAL	1319	100	129	100	41	100	1489	100

Table 2. Relative distribution of glioblastomas in the four clusters.

CLUSTER	PG		CGC		GS		TOTAL	
	Nr	%	Nr.	%	Nr.	%	Nr.	%
C1	542	97.13	15	2.69	1	0.18	558	100
C2	461	83.51	58	11.11	3	0.57	522	100
C3	235	95.92	9	3.67	1	0.41	245	100
C4	81	49.39	47	28.66	36	21.95	164	100
TOTAL	1319	88.58	129	8.66	41	2.75	1489	100

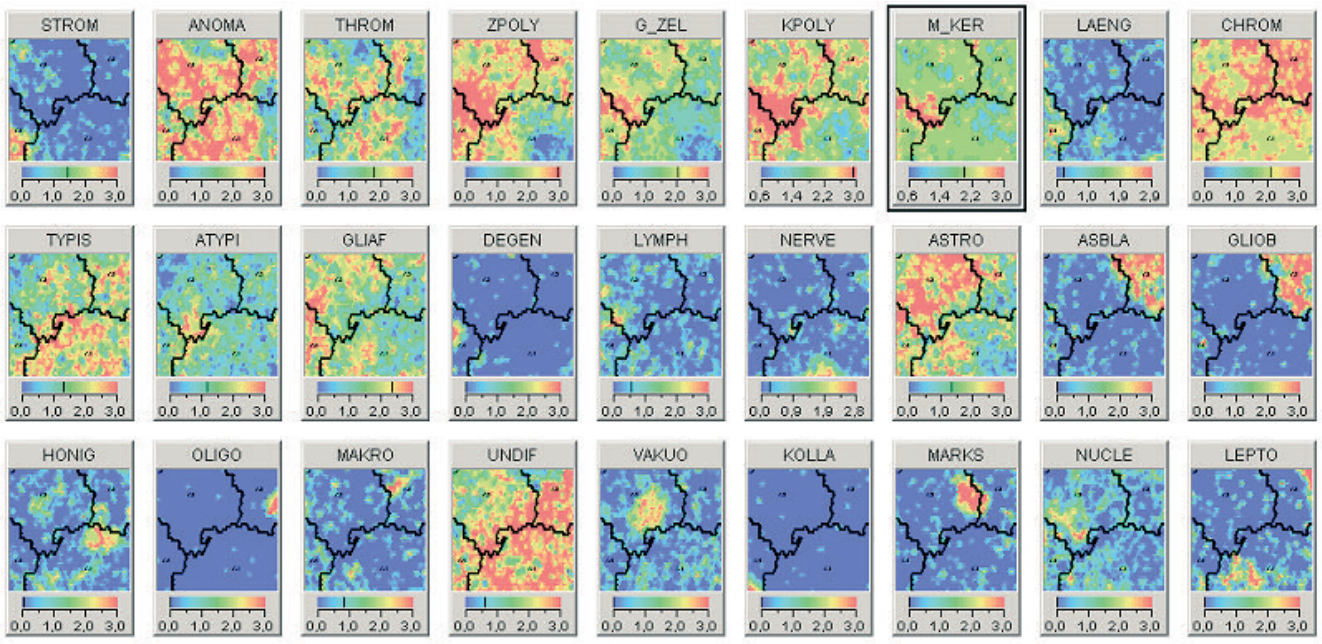

Fig. 3. Significant component windows and separators for four clusters. In all windows, blue signifies total absence of the feature and red its strong presence. Cluster C1 showed cases with a lot of typical mitosis (TYPIS), dedifferentiated cells (UNDIF), the presence of preexistent nerve cells (NERVE), and infiltration of the leptomeninges (LEPTO). Cluster C2 showed GB with a lot of astrocytes (ASTRO), Vacuolization (VAKUO), the presence of myelin between the tumor cells (MARKS) and of prominent nucleoli (NUCLE). Cluster C3 shows GB with the presence of an important amount of astroblasts (ASBLA), and glioblasts (GLIOBL). A small subgroup showed the presence of oligodendroglia. Cluster C4 showed GB with a strong presence of stroma (STROM), oblong nucleus (LAENG) and collagen fibers (KOLLA).

Another small subcluster (bottom left) was built with prominent nucleoli (NUCLE). *Cluster C2* was divided into three subclusters with different amounts of typical (TYPIS) and atypical mitoses (ATYPI), and vacuolization of the tumor (VAKUO). A subcluster contained many intratumoral myelinated fibers (MARKS). In *cluster C3* a subcluster contained many astroblasts and glioblasts and a second subcluster many oligodendroglial cells. *Cluster C4* included two subclusters with a different quantity of stroma (STROM), typical mitoses (TYPIS) and collagen fibers (KOLLA).

Discussion

The self-organizing map (SOM) (Zell, 1994; Kohonen, 1995, 1997) is a neural network algorithm based on unsupervised learning, which allows the visualization of complex multidimensional data. Fifty histological features as well as age and gender are the data on which our study was based. The SOM combines vector quantification and projection, which together provide a map of the data giving a visual insight into their properties. SOM is formed of input-neurons, in our study these were 1489 GB containing 50-dimensional vectors (the 50 values pertaining to each patient). These neurons communicate with the neurons of the two-dimensional hexagonal grid of the map which, in our study, was constituted by 2021 nodes. During the training, all the input-vectors together are compared with the weight-vectors. The neuron of the map that has the smallest distance to the input-vector is the winning neuron. This is the way to calculate the morphological and immunohistological likeness (similar colors) among the diverse GB. “Similarity" indicates, thus, a mathematical distance. On the other hand, two-dimensional maps show clear similarities with histological slides and therefore the data visualized in this form are "more familiar" to pathologists and radiologists than columns of numbers or even statistical data. The more common visualization of the SOM, as in our study, takes the form of a two-dimensional grid (Zell, 1994; Vesanto, 1999). These maps indicate the density of the weight-vectors of the distribution of the GB and they adapt themselves in the input-area.

The incidence, age and sex distribution of GB in our series are similar to those of other reported series (Zülch, 1986; McLendon et al., 1998). In our study four clusters or groups of GB could be recognized using SOM with a maximal significance of 52 (minimal = 0, maximal value 60) without previous classification. Recognition of clusters underline the fact that the different GB are a heterogeneous histological group of tumors. However, some histological components are determinant for significant clustering. The distribution of the different phenotypes or clusters found in our study perhaps could also indicate the distinct molecular alterations of the GB (Kornfeld, 1986; von Deimling et al., 1993; Hayashi et al., 1997; Meyer-Puttlitz et al., 1997; Duerr et al., 1998; Schmidt et al., 2002). In the 2000 WHO classification (Kleihues and Cavenee, 2000) glioblastoma multiforme includes only the variants of

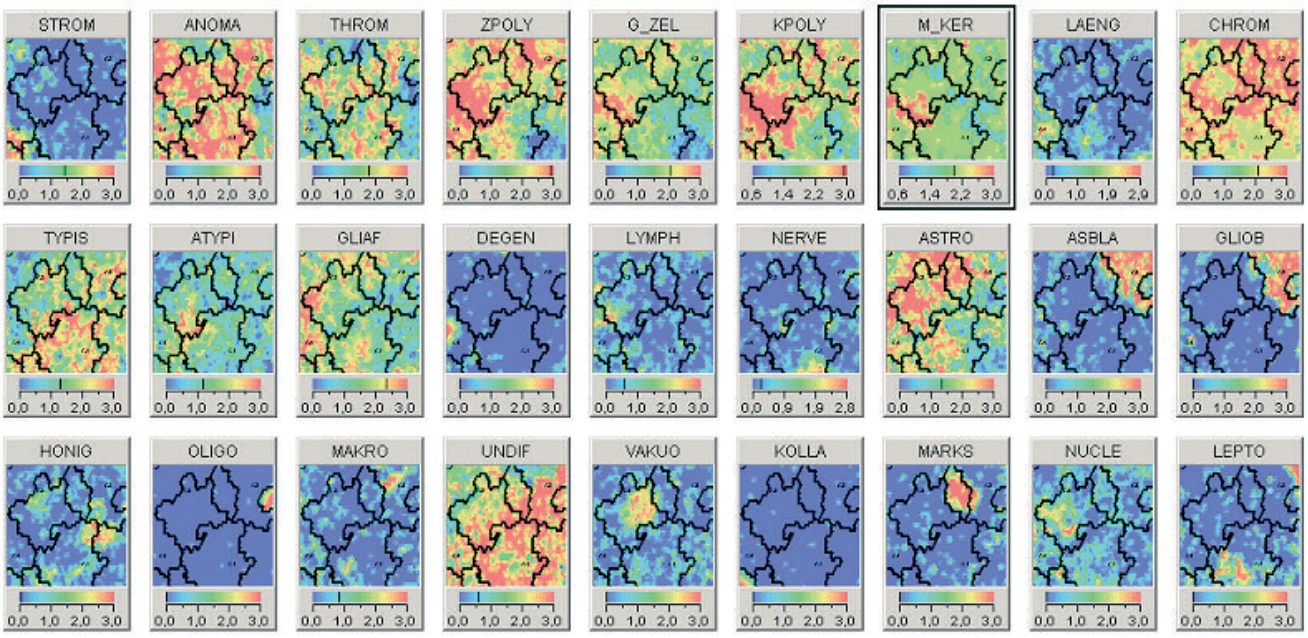

Fig. 4. Significant component windows and separators for 10 clusters with a significance of 29. Clusters C1 and C2 contained 3 subclusters. Clusters C3 and C4 contained 2 subclusters.

GCG and GS, but other variants were reported by Kornfeld (1986), composed of granular-appearing cells, similar to the "isomorphic glioblastoma" described by Río Hortega (1932) and Polak (1966). Cluster C1 may correspond to PG and cluster C2 to GCG. Cluster C3 containes two subclusters with a lot of astroblasts and glioblasts, and with oligodendroglial cells which may correspond to different groups of "isomorphic glioblastoma". For the Ward- and SOM-Ward-cluster method, a separator is the line that is drawn between two nodes if they are in different clusters and the building of a cluster depends on several components or histological features. Some features like polymorphic cells (ZPOLY) and polymorphic nuclei (KPOLY) belong to clusters C1, C2 and C4. However, the presence in GB of many astroblasts, glioblasts and oligodendroglial cells is responsible for the formation of new possible biological groups of GB demonstrated by cluster C3 with a its subcluster. This could be have important clinical implications, because GB with an oligodendroglial component are associated with longer survival than classical GB (Donahue et al., 1997).

A second biological group of GB could be found in one subcluster of C1 (Fig. 4, left bottom) that included cases with scanty anomalous vessels (ANOMA), few thromboses (THROM) as well as polymorphic cells (ZPOLY), small cells (G-ZELL), and a small quantity of polymorphic nuclei (KPLY). The subcluster may correspond to the "isomorphic glioblastoma" of Del Río Hortega (1932) and Polak (1996) and was morphologically similar to the granular cell glioblastoma described by Kornfeld (1986). Small subclusters with a little mathematical significance may show a small group of GB with specific biological features. A possible new group of GB could be represented by the subcluster of C2 with the component MARKS (Fig. 4). These GB were characterized by the presence of many intratumoral preexistent myelinated axons which were observed by strongly infiltrating GB.

The histological cluster C4 correlated with the diagnosis of GS corresponding to the WHO-classification. 87.80 % of all gliosarcomas of our series were included in this cluster (Table 1). Furthermore, strong values of the cluster-components namely stroma (STROM), oblong nuclei (LAENG), collagen fibers (KOLLA) and degenerative changes of tumor cells (DEGEN) showed typical components of this group (Figs. 3, 4). In addition, 37.72% of GCG were included in this cluster. Foci of lipid degeneration may be conspicuous in GCG (Gherardi et al., 1986). In addition, the development of reticuline fibers and other connective tissue may be extensive and occasionally interpreted as sarcomas (McLendon et al., 1998).

The SOM allowed, in our study, a realistic histological classification comparable to the actual WHO-classification (Kleihues and Cavenee, 2000) with the advantage of providing a visualization of multidimensional histological features. New histological subclusters or groups of GB could be detected. Comparing new data with the map helps classify the data and gives an indication of whether the new data belong to the same data distribution or cluster or not. By using the qualitative information given by visualization, sub areas and sub spaces can be selected from the data and a quantitative analysis of these performed rather than doing so blindly on the entire data. Thus, after filtering, the data can be reanalyzed. With SOM we can learn to discriminate, discard and delete data, select histological and clinical variables that are meaningful, and so influence the outcome and patient management. New or not foreseen subclusters as in C2 and C3 may initiate the investigations as to whether using statistical methods could have a practical significance, or whether the GB or clusters could build a group with specific genetic alterations. In a previous study (Iglesias-Rozas et al., 2000), using SOM techniques, a cluster of GB with long survival was found. These patients were treated with radiotherapy and chemotherapy. Other features such as sex, age and coefficient of malignancy (Iglesias et al., 1983, 1987) were not important for the grouping in the present study.

Neuronal networks, and especially the SOM, do not substitute the traditional statistical studies nor the actual classification; however, the visualization and comparison of the different variables with the help of SOM-maps allow an efficient multifactorial analysis of the parameters included in the study. Histological clustering strategy has been used in children with infratentorial neuroglial tumors (Gilles et al., 1998) to identify homogeneous histological subsets. In the future prospective studies with the help of SOM-nets in patients with brain tumors would allow a more specific evaluation of new predictive factors, genetic findings, treatments and a more effective comparison with established tumor managements.

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