School of Electrical, Electronic and Computer Engineering

MSc Communications and Signal Processing

Literature Review

An Artificial Neural Network-based evaluation of cellular and molecular markers for the assessment of potential indicators for breast cancer survival analysis

By

Shirin Ameiryan Mojarad

Std No. 069145621

Supervisor: Prof. S.S.Dlay

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Abstract

The use of artificial neural networks (ANN) in cancer prognosis application especially in breast cancer has become an interesting area for researches due to the fast growth of breast cancer among women. The objective of this literature is to investigate the potential of some biomarkers of breast tumours to predict the breast cancer progression and survival. To accomplish this objective, a review of more than 25 technical papers has been completed to depict the superiority of new approaches in ANNs, especially feedforward multilayer percepron ANNs, over conventional analysing methods suffered from low accuracy.

The aim of the study is to analyze some tumour biomarkers using an artificial neural network to determine the potential of these indicators to predict the 5 year survival in patients involving breast cancer and presence of metastatic tumour in the regional lymph nodes. A further objective of the study is to evaluate the impact of individual markers on predicting outcome in both cases.

CONTENTS

Acronyms

ANN	Artificial Neural Network
AUC	Area Under the Curve
BP	Backpropagation
ER	Oestrogen Receptor
FF	Feedforward
ICM	Image Cytometry
MLP	Multi Layer Perceptron
MSE	Mean Square Error
MLFFBPNN	Multi Layer Feedforward Back Propagation Neural Network
ROC	Receiver Operating Characteristics
PR	Progesterone Receptor
SPF	S-Phase Fraction

1. Introduction

Breast cancer affects a significant number of women every year and is the major cause of cancer death amongst women. Statistics illustrate that among every ten women, one woman is involved with breast cancer [1]. There are more than 35000 women diagnosed with breast cancer in the UK every year and it has also been estimated that nearly 1000 women die every month due to breast cancer [2]. Therefore, breast cancer prognosis has become an area of interest in the recent years. Prognosis of breast cancer and defining an adjuvant treatment strategy is highly correlated with the state of tumour progression in axillary lymph nodes. A proportion of women receive unneeded axillary surgery because of wrong diagnosis of nodal metastasis. For instance the risk of relapse for a patient with negative axillary lymph node and a tumour sized 1-2 cm is about 20-30% [3]. Most patients in this stage receive adjuvant therapies whilst 70% of them do not actually need it as their disease is already treated. It is also very important not to miss many node-positive cases since patients with early stage breast cancer can highly benefit from an adjuvant treatment and surgery if they are diagnosed in time. Given that the accurate treatment principally depends upon the status of tumour progression, many factors have been investigated in order to define the disease progression in breast cancer.

Fisher et al. [4] showed that the number of involved nodes and the metastasis to axillary lymph nodes are related to the prognosis of breast cancer. He also used tumour size and cellularity, its location, histological differentiation and the status of steroid and growth factor receptors as markers of tumour progression. Unfortunately the histological information is not an effective factor for assessing individual risk and preventing unnecessary therapy [3].

Since there has been an observation of microscopic metastasis in early-stage breast cancer in a significant number of patients, novel prognostic markers have become the area of interest for breast cancer prognosis. It has been shown that some biomarkers including cellular and molecular markers can be used as independent or non-independent prognostic indicators. A large variety of these biomarkers and their prognostic value in different kinds of cancer have been explored to make an accurate prognosis about tumour progression so as to help the physicians to identify the best therapy for the disease.

The method used for analysing the tumour markers in order to identify their prognostic value is also an important issue. Because of the fast growth of breast cancer among women and ever increasing number of women involved with it, vast researches have been done in this area and a large number of features have been detected to be effective in breast cancer. Along with the high volume of data, the nonlinear nature of the interaction between these features has caused the traditional statistical analysis techniques to be inefficient in classifying this large amount of data.

Moreover, the increased number of patients and high costs of cancer treatment beside the undesirable effects of therapies makes an accurate inexpensive way of prognosis highly in demand. In 1980s, neural network was found to be a powerful tool in modelling complex systems in compared with traditional statistical methods [5]. Since then, it is widely used in medical applications such as radiology, neurology and cardiology; but perhaps it has been mostly applied in the area of oncology especially for assessing the prognostic value of different biomarkers and risk factors in defining the state of tumour progression.

This research is based on a set of clinical data which contain nodal status of patients, some information about 5 year survival with or without metastasis and the data relating to some biomarkers of the tumour. The following aims and objectives are pursued in this study:

Aims

- investigate the predictive accuracy of effective factors in breast cancer prognosis

- Predicting the 5 year survival in breast cancer in terms of alive, dead or alive with metastasis
- Defining the nodal status of the cancer which contains positive or negative metastasis status

Objectives

- Study different types of tumour biomarkers which are effective in breast cancer prognosis
- Explore the effect of individual biomarkers on the prediction outcome
- Investigate the construction of Multi Layer Feedforward Backpropagation Neural Network (MLFFBPNN)
- The software implementation of ANN to evaluate its reliability and accuracy in cancer prognosis

A general overview of the project is depicted in figure1.

In this literature, some general information about some biomarkers, commonly used in breast cancer prognosis, is reviewed. Then we will go through the main concept of Artificial Neural Networks (ANN), Multi Layer Perceptron (MLP) and Feedforward (FF) ANNs. Finally, a literature survey of the application of different tumour biomarkers and ANN in breast cancer prognosis and the previous studies in this subject is covered.

2. Biomarker definitions

Breast cancer is known as a multifactorial disease caused by genetic and non-genetic features. Many cellular and molecular markers including different genes, steroid hormone receptors, tumour and histological grade, DNA ploidy, S-Phase fraction (SPf), tumour size and cell cycle distribution have been studied to define their value in the prognosis of the tumour progression in breast cancer. These prognostic indicators are divided into three groups namely, traditional, cellular and molecular markers. Each group include a number of markers which early breast cancer prognosis and diagnosis is based on them.

Although in most studies only a subset of these markers is analyzed, in this research, all of the markers are used as the input variables of NN to investigate their predictive accuracy in breast cancer prognosis.

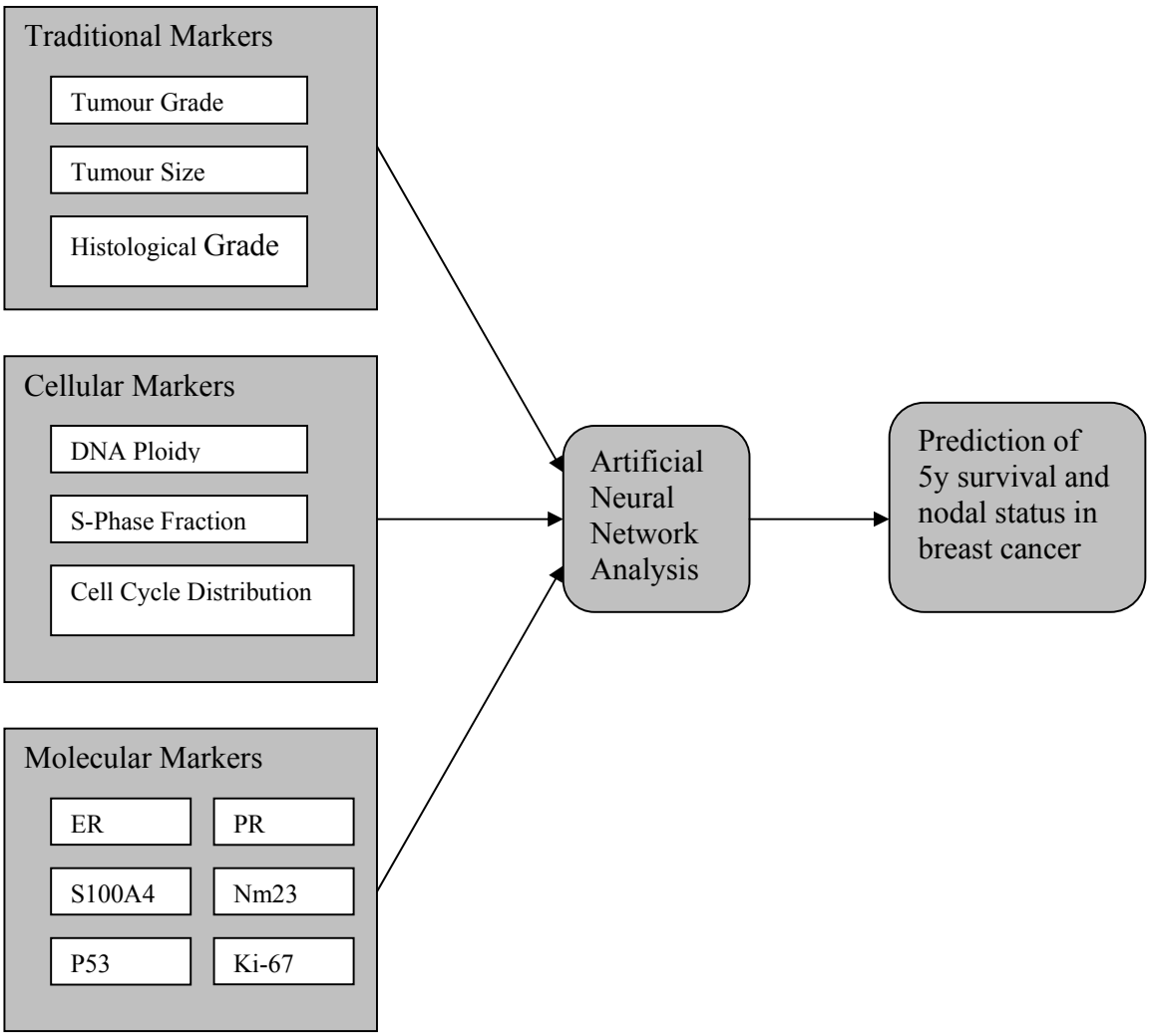

Figure1. An overall view of the project

2.1 Traditional markers

The traditional markers are those markers which are typically assessed visually by an expert using microscope or physical examination. Age, tumour size and grade and histological grade are some traditional prognostic factors used in breast cancer prognosis.

2.1.1 Age

The role of age as a prognostic factor in breast cancer studies is evidently verified. Although the nature of interaction between age and cancer prognosis has not been clearly defined so far, there is a sound agreement about the higher risk of undesirable results in the younger patients.

2.1.2 Tumour size

Tumour size is conventionally determined by measurement of the three largest dimensions of a tumour. The determination of the size of the tumour can be done alternatively by measurements in histology.

2.1.3 Tumour grade

Tumour grade is derived from the spatial characteristics of the tumour tissue including the morphological properties of the cancer cells nuclei, distribution of them and the expression of the cancer associated surface receptors [6].

2.1.4 Histological grade

A modified form of The Bloom and Richardson grading is currently applied for determining the histological grade of the tumour. In this approach, three factors including the degree of tubule formation, mitotic index and nuclear pleomorphism are used for grading the tumour.

2.2 Cellular Markers

Cellular markers are also identified as cytological factors since they are measured by the means of image cytometry (ICM). Theses markers include S-Phase fraction (SPF), DNA ploidy and cell cycle distribution.

2.2.1 DNA Ploidy

DNA aneoploidy is a state in which abnormal sets of chromosomes exist within the nucleus and is considered as an indicator of tumour malignancy. Many studies have investigated the role of DNA ploidy of cancer cells in cancer prognosis. The results demonstrate that it is highly associated with relapse of the disease [7], reduced survival time [8] and metastasis to regional lymph nodes and early death [9].

2.2.2 S-Phase Fraction (SPF)

S-phase fraction measurement indicates the percentage of cells in the stage of DNA replicating in cell cycle. The size of the SPF is closely related to cell features and it is a validated marker for estimating the proliferative rate of tumour cells [10].

2.2.3 Cell Cycle Distribution

The pattern of cell cycle distribution is defined by the G0G1/G2M ratio (ratio of the number of the cells in G0G1 phase over the number of the cells in G2M phase) which is measured by ICM [1].

2.3 Molecular Markers

Some of the molecular markers known to have prognostic value in tumour progression in breast cancer include genes S100A4, nm23, Ki-67, P53, histological grade, tumour size and steroid hormone receptors including Oestrogen and Progesterone receptors (ER and PR).

2.3.1 S100A4

S100A4 is known as metastasis promoter gene [11]. S100A4 is a calcium binding protein which is present in normal breast tissue. Presence of S100A4 in breast cancer cells shows a high reduction in patient survival [12]. This diminution in survival rate in presence of the S100A4 is because of its stimulus impact on tumour metastasis and

malignancy; thus, existence of S100A4 is highly correlated with the presence of breast cancer [13].

2.3.2 Nm23

Nm23 is known as a metastasis suppressor gene in breast cancer [11]. Nm23 –H1 and nm23-H2 are its two homologues genes that have been also found to be correlated with low rate of tumour metastasis [14]. Many studies suggest the association of nm23 and low potential of breast cancer metastasis. Nevertheless, this connection has not been proved in some researches [11].

2.3.3 Ki-67

Ki-67 is considered as a marker of proliferation in early-stage breast cancer [3]. This marker is actually a nuclear antigen which can be found in the proliferative phases of the cell cycle (i.e. G1 phase, S phase, G2 phase and M phase) but not in the resting phase of the cells (G0 phase). It has been found that the tumours with high amount of Ki-67 in more than 50% of the cells have a high risk of recurrence [15].

2.3.4 P53

P53 is known as a tumour suppressor protein [12]. The synthesis of p53 is increased when DNA is damaged and its accumulation will lead to stop replication of DNA and cell division until DNA is repaired. In the cases that DNA is unrepairable, p53 may cause the cell death. Thus in the absence of p53 (when p53 is mutated or lost), the damaged DNA would be replicated and the mutation will be passed on to daughter cells. P53 expression is also considered as a preventing factor in tumour formation by preventing the cell cycle progression especially in G1S and G2M phases. Thus, high expression of p53 can be interpreted as a good guide to survival in breast cancer patients.

2.3.5 Steroid Receptors

In many studies, It has been proved that the status of hormone receptors of breast cancer cells can be used as useful information for cancer prognosis and treatment [1, 3, 11]. The steroid receptors considered in this study include Oestrogen Receptor (ER) and Progesterone Receptor (PR).

Oestrogen is a hormone with growth stimulating ability in a variety of target tissues. It binds to oestrogen receptors which are then transmitted to the nucleus where they instigate responsive genes transcription and lead to appropriate psychological function. Oestrogen and progesterone hormones can initiate the transcription of some target genes related with cell differentiation and proliferation [16]. Since anti-oestrogen therapy is not effective in ER negative tumours, the tumour grows rapidly leading to poor results for patient; thus, the absence of ER in breast cancer is considered as a sign of poor prognosis. ER absence in breast cancer is caused by ER gene silencing resulting from hypermethylation [11].

The role of the PR positivity in breast cancer is less significant. Normally, ER positive cancers are also PR positive, but there would be a poor prognosis for PR positive tumours that are not ER positive.

3. Background

Techniques of data collection and analysis in cancer prognosis have noticeably improved in the recent years. As mentioned previously, in this research it is aimed to analyse tumour biomarkers by the means of an ANN to define the prognostic value of theses features in breast cancer. Thus, the main concept of ANNs, Multi Layer Perceptron (MLP) and Feddforward Backpropagation NNs, which is used in this project, is reviewed; after that, the related works in this field is explored.

3.1 Artificial Neural Network

ANN basic form and the preceptron concept were firstly introduced by McCulloch and pitts in early 1940s [17]. The structure and function of ANN resembles the animal brain,

in that they include a massively parallel architecture, but in a relatively small scale. Brain is made up of neurons whereas ANN is based on layers of computing nodes. Perceptron is referred to a system consisting of an artificial neuron and input data (stimuli). A multilayer perceptron neural network, MLP, consists of many nodes which form layers. The simplest form of trainable perceptron network, first developed in 1959 [18] and 1960 [19], encompasses two layers of nodes namely input and output layer. In this case, a mapping between the input data and output signal is established by assigning weights to the input numerical data during training. More complicated MLPs which are commonly used consist of some hidden layers in addition to the input and output layer (figure2). The connection between these layers is defined by weights which are assigned in a supervised learning process so that the neural network will be able to classify the pattern supplied by the user. This can be done via backpropagation algorithm in which a network is trained based on the sampled data so that the network would respond correctly.

Training algorithm consists of building a predictive model that minimizes error when the network's output is compared with a known output. Afterwards, the network would be validated with a part of the available data and its performance in terms of success in arriving at a correct prediction related to each input would be evaluated based on the Mean Square Error (MSE) and the Area Under the Curve (AUC) of Receiver Operating Characteristics plot (ROC).

Three non-overlapping groups of data are used for training an ANN. These groups include training set, testing set, and verification set. The training set is employed for adjusting the weights during training while the testing set is applied to define when to stop training because otherwise, the learnt features by the ANN may not be present in other cases (which have not been used in training process). Afterwards, the competency of the achieved ANN would be validated by the verification set in which a set of non-trained data will be used as the input of the ANN and the outcomes of ANN will be compared with the actual results.

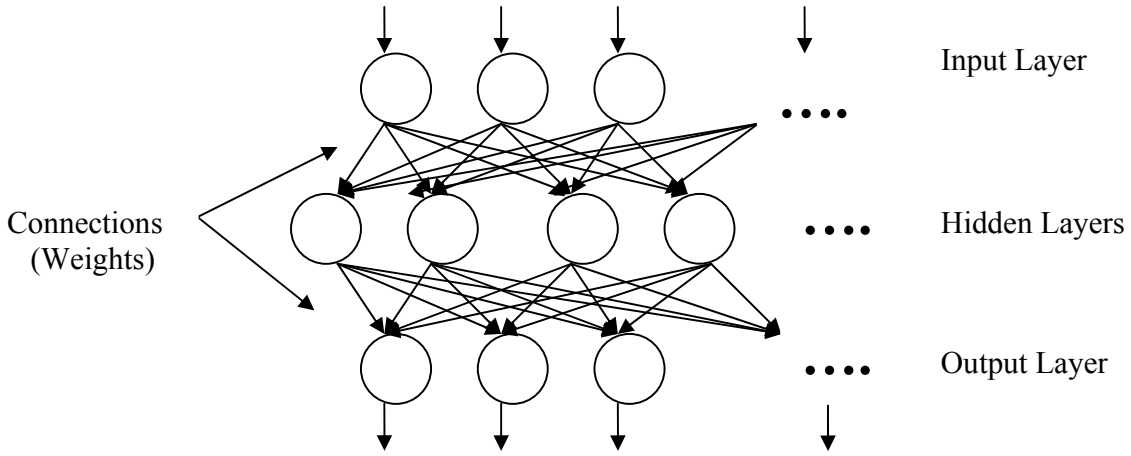

Figure2. Multilayer Perceptron

3.1.1 Feedforward artificial neural networks (FFANN)

FFANNs are commonly used for classification tasks, especially in tumour prognosis, staging and survival prediction [19]. Typically, FFANNs are organized as a set of interconnected hidden layers of artificial nodes in which each neuron receives its inputs from the nodes of the previous layer (figure2). The output activation by a numerical value is called the "firing" process. There is a level for output activation in MLP which is described by a function called "activation" or "squashing" function. There are different types of activation functions but sigmoid function [S(x)] is the most frequent one which is defined as:

$$S(x) = 1/[1+\exp(-x)] \tag{3.1}$$

where x is the input of the activation function (figure3). In a biological model, this sigmoid function can be explained as the sum of the products of the incoming weights with their associated activation levels expressed by S_m:

$$S_m = \sum_{n=0}^{j} W_{mn}\, a_n \tag{3.2}$$

where W_{mn} is the incoming weight for the nth unit, a_n is activation value of the nth unit and j is the number of connections between nth and mth units.

Biomedical studies generally employ three layer networks with an input layer, one hidden layer and an output layer in which the layers are fully interconnected [19]. In MLP, a weight is assigned to each connection; thus equation 3.1 becomes:

$$a_{m,i+1} = 1/[1+\exp(-\sum W_{mn,i}\, a_{n,i})] \qquad (3.3)$$

where $a_{m,i+1}$ is the activation value of unit m from the layer i+1, $Wmn_{,i}$ is the connection weight between the nth and mth nodes of ith and i+1th layers and $a_{n,i}$ is activation value of unit n from the ith layer. In a three layer MLP, two types of connections including i=1,2 exit.

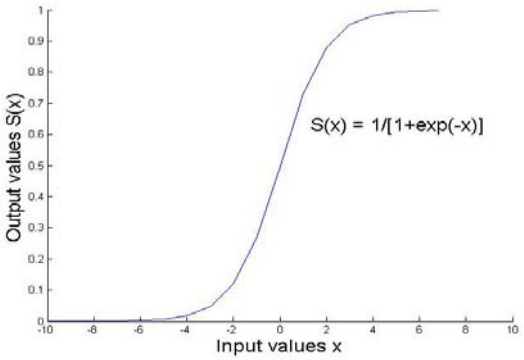

Figure3. Sigmoid function S(x) used as an activation function for MLP

There are some ways to find the optimal number of hidden neurons such as cross validation, pruning or bootstrapping but still the most popular approach is trial and error.

In cancer survival analysis, the prognostic role of the different markers in determining the patient outcome can be achieved using a neural network set by finding the relation between the predictive values and rates of survival [20].

3.2 Related studies

Many researchers have analysed the prognostic value of different biomarkers in breast cancer prognosis through different methods. These methods can be classified in two general groups namely, machine learning methods and statistical methods. Since cancer prognosis is a complex process involved with many non linear factors, higher accuracy

can be achieved via machine learning techniques. Meanwhile, most studies concur with the high efficiency of ANNs as a classification and survival prediction technique in different biomedical systems.

3.2.1 Statistical methods

Conventional statistical methods used in cancer prediction include TNM staging, classification, principle component analysis, cox's proportional hazards and logistic regression (LR). TNM staging has been used as a universal model for cancer prognosis since 1960's. Ever since, many new prognostic factors for breast cancer have been detected, but integrating the new factors into TNM stage model is not feasible. Therefore, this model is not very accurate and it is not possible to improve its accuracy [21]. In conclusion, reliable results can not be achieved by conventional statistical methods in cancer prognosis compared to ANNs [22, 23].

3.2.2 Machine learning methods

Bayesian and nearest neighbour classifiers, decision trees, fuzzy logic and ANNs are categorized as machine learning classifiers [24]. Some studies propose neuro-fuzzy modelling as a more accurate method for relapse prediction in cancer than NNs [25]. On the other hand, there are some conclusions about the same accuracy of fuzzy and NN in the case of medical outcome prediction [21]. Furthermore, some literatures suggest a hybrid method consisting of ANN and fuzzy logic for a more efficient and accurate prognosis in breast cancer [26].

3.3 Literature review of ANNs in breast cancer prognosis

ANN models are able to classify the input factors and produce outcomes based on nonlinear regression and discrimination. In the case of adding more variables to NNs, it is possible to improve their accuracy in compare with statistical methods. A great variety of ANNs have been detected and used in cancer prediction but it has been proved that only a few of them are effective in cancer prediction, among which, conjugate gradient NN, cascade correlation NN, backpropagation NN, and probabilistic NN can be mentioned as some frequent networks used in cancer prognosis. Meanwhile, reliability and superiority

of MLFFBPNN (Multi Layer Feedforward Back Ppropogation Neural Network), proposed by De et al [27], is confirmed as a feature evaluation index compared to other types of NNs.

Naguib et al. [28] have conducted a research for the nodal metastasis detection in breast cancer using neural network techniques and have concluded that neural networks can provide reliable indicators for lymph node status using merely basic measurements of breast tumour. Thus, it is suggested that the addition of less common markers can improve the accuracy. In another research it has been proved that ANN analysis is an effective technique for predicting the recurrence probability of breast tumours but its results are not as clear for the nodal metastasis status. In addition, Biganzoli et al. [29] has included that feed forward artificial neural networks provide a flexible non-linear model of survival data.

Grey et al. [11] have investigated the prognostic value of some molecular and cellular markers including tumour promoter and suppressor genes S100A4 and nm23, oestrogen and progesterone receptors and tumour grade and size. Analyzing these markers by an ANN, it was shown that tumour grade and size are poor predictors in the case of predicting tumour metastasis to the regional lymph nodes. It was found that the relative expression of the S100A4 and nm23 genes is the most effective indicators of the nodal status. ER and PR have been found to improve the prediction in conjunction with tumour grade and size; nevertheless, there may be overlap between these steroid receptors and molecular markers. In this study ANN was suggested as a powerful technique in predicting the potential of breast cancer metastasis to the axillary lymph nodes.

Although recent researches have proven the immense ability of ANNs in enhancing the prognostic accuracy in oncology comparing with more traditional statistical methods, it was suggested by some studies that they may also lead to inaccurate findings as a result of variety of factors such as complex architecture, need of modification of a large array of variables and their random initialization [2]. Moreover, the obtained results by an ANN can be interpreted differently because of lacking any level of certainty or degree of confidence correlated with them.

In spite of diverge opinions about the effectiveness of ANN in cancer prognosis yet, it can be referred to as a reliable technique in the case of cancer prognosis.

4. Discussion

Vast researches conducted in the case of breast cancer prognosis have leaded to identification of many new prognostic markers. However, besides exploring new markers, finding the relationship between the new markers to those previously used, and the additional information they provide is also very important. Therefore, the need for an analysing system being capable of giving a reliable prediction for cancer progression on the basis of the tumour markers and define the predictive accuracy of these markers is obvious. Many studies have confirmed the effectiveness of ANNs in the area of cancer diagnosis and prognosis.

In this research, a MLFFBPNN model is employed to define the predictive accuracy of the features or subsets of features in breast cancer prognosis in terms of the 5 year survival and nodal status. For the purpose of simplicity, tumour biomarkers are classified into three groups namely traditional, cellular and molecular markers, each group including some effective markers in breast cancer prognosis. In the first step, these markers will be assessed in terms of three outputs for five year survival (patients alive, dead and patients alive with metastasis to the regional lymph nodes). In the second step, features are evaluated in terms of two outputs regarding nodal status (patients with metastatic or non-metastatic tumours).

To achieve these aims, the data set has been divided into two subsets. One set of data is used for training the neural network and the other set would be utilized for validating the ability of the network for 5 year survival and nodal status prediction. The study starts with employing all the biomarkers as the input data and then biomarkers will be partitioned into subsets for which the NN will be trained. Moreover, in this way the prognostic value of the individual biomarkers in conjunction with each other would be defined.

It is expected to obtain a set of markers for which the neural network analysis would give an accurate and reliable result for 5 year survival analysis and nodal status in breast cancer.

5. Conclusion

Early diagnosis and identifying the state of tumour progression accurately has a great impact on patient treatment and management. There are some non invasive methods for prognosis of breast cancer such as clinical/physical examination, mammography, etc. however these methods are not trust worthy especially in small tumours which are not palpable.

ANN is not only a reliable technique for obtaining and analysing prognostic factors, but it is also an economical way for retrieving data for both the patients in terms of rate of morbidity and stress and to the screening centres, clinics and health service in running expensive schemes. Neural network can easily create a nonlinear interaction between variables and provide a more efficient model for predicting the survival time in breast cancer patients in compare with traditional methods such as LR which suffer from poor reliable conclusion in the case of cancer prognosis. In the case of machine learning algorithms, in spite of the high accuracy achieved by hybrid algorithms and neuro-fuzzy modelling, ANN is still a good choice among other machine learning algorithms. Consistency and reliability in terms of decision making are two significant characteristics of ANNs [30] which satisfy their use as robust analyzing techniques in biomedical applications.

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