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1.1 Introduction:

3D printing, also known as additive manufacturing, is the process of creating three-dimensional objects from digital files by adding layer upon layer of material. It is a revolutionary technology that has transformed the way we think about manufacturing, design, and engineering. The history of 3D printing dates back to the early 1980s when the first 3D printer was invented. In this essay, we will explore the history of 3D printing and its evolution over the past few decades.

1.2 The Early Days:

The first 3D printer was invented by Chuck Hull in 1983. He called his invention stereolithography (SLA) and it used a process called photopolymerization to create objects layer by layer. The SLA process involved a laser that would selectively harden a liquid resin, which would then form the object. Although this process was slow and expensive, it laid the foundation for future developments in 3D printing.

In the late 1980s and early 1990s, 3D printing began to attract attention from the manufacturing industry. This was due to the development of new technologies that made the process faster and more affordable. One of the most significant developments during this time was the invention of fused deposition modeling (FDM) by Scott Crump. FDM used a process of extrusion to create objects by melting and depositing material layer by layer. This technology was more affordable and allowed for the creation of more complex objects.

1.3 Rapid Prototyping:

In the 1990s, 3D printing began to gain popularity as a tool for rapid prototyping. This is because 3D printing allowed engineers and designers to quickly create physical prototypes of their designs. This was a significant improvement over traditional prototyping methods, which were slow and expensive. Rapid prototyping allowed for faster design iterations, reduced time to market, and ultimately lower costs.

1.4 Commercialization:

In the early 2000s, 3D printing began to move from the realm of rapid prototyping to commercial production. This was due to improvements in technology that made 3D printing faster, more reliable, and more affordable. In addition, the availability of more advanced materials such as metal and ceramics expanded the range of applications for 3D printing.

1.5 Today:

Today, 3D printing has become a mature technology that is used in a wide range of industries, including aerospace, automotive, medical, and consumer products. 3D printing has allowed for the creation of complex and lightweight parts that would have been impossible to

produce with traditional manufacturing methods. In addition, 3D printing has reduced waste and lowered the environmental impact of manufacturing by using only the necessary amount of material. In conclusion, 3D printing has come a long way since its inception in the early 1980s. From its beginnings as a slow and expensive technology, 3D printing has evolved into a mature technology that is used in a wide range of industries. The future of 3D printing is bright, as new materials, faster printing speeds, and improved software are constantly being developed. As 3D printing continues to improve, it has the potential to transform the way we think about manufacturing and production.

2. The Usage of 3D Printing in Medicine

2.1 Medical Applications:

3D printing is also being used in the medical field. One of the most significant applications of 3D printing in medicine is the creation of customized implants and prosthetics. 3D printing allows for the creation of highly personalized and precise medical devices that are tailored to a patient's specific needs. In addition, 3D printing is being used to create models for surgical planning and training, which can lead to better outcomes and reduced surgery times.

2.2 Education:

3D printing is also being used in education. 3D printing allows students to bring their ideas to life and create physical objects that they can hold and interact with. This can be a powerful tool for teaching concepts such as geometry, physics, and engineering. In addition, 3D printing can help students develop critical thinking and problem-solving skills by challenging them to design and create their own objects.

2.3 Conclusion:

In conclusion, 3D printing has a wide range of applications, from rapid prototyping to commercial production, to medical applications and education. 3D printing has transformed the way we think about manufacturing, design, and engineering. As the technology continues to improve, it has the potential to revolutionize many industries and create new opportunities for innovation. As 3D printing becomes more accessible and affordable, it is likely to become an increasingly important tool for businesses, educators, and individuals alike.

The usage of 3D printing has gained popularity in the medical field in recent years. One of the areas where 3D printing is being utilized is cardiology. 3D printing has emerged as a valuable tool for diagnosing and treating cardiac diseases by providing accurate anatomical models of the heart.

Cardiac surgeons and interventional cardiologists use 3D printing to create replicas of the heart, which allows them to have a better understanding of the patient's anatomy before surgery. These 3D printed models can help in the planning and execution of complex cardiac procedures, such as valve replacement, coronary artery bypass grafting, and stent placement.

Additionally, 3D printing is being used to create customized cardiac devices, such as prosthetic valves, pacemakers, and stents. These devices can be precisely tailored to fit the patient's specific anatomy, improving the efficacy and safety of the procedure.

One of the primary benefits of using 3D printing in cardiology is that it reduces the risk of surgical complications. The ability to create an accurate 3D model of the patient's heart allows the surgeon to plan the surgery in advance, reducing the risk of complications during the procedure. This leads to better outcomes and faster recovery times for patients.

Another benefit of 3D printing in cardiology is that it allows for better communication between the medical team and the patient. The 3D model can help the patient understand their condition and the procedure better, which can improve patient satisfaction and outcomes.

In conclusion, 3D printing has revolutionized the field of cardiology by providing accurate and customized anatomical models, improving surgical planning and reducing complications. As the technology continues to advance, it is likely that 3D printing will become an even more valuable tool for diagnosing and treating cardiac diseases.

3. Future of 3D printing Usage in Cardiovascular Health

The usage of 3D printing in medicine, including cardiology, is still in its early stages, but there is a vast potential for its future applications. Here are some potential areas where 3D printing could be used in medicine and cardiology in the future:

3.1 Personalized implants and devices: 3D printing allows for the creation of highly personalized implants and devices that are tailored to the patient's specific needs. In the future, it may be possible to create customized cardiac devices, such as heart valves or pacemakers, that are perfectly matched to the patient's anatomy.

3.2 Bio-printing: Bio-printing is the process of creating 3D structures made of living cells. In the future, it may be possible to use bio-printing to create functional cardiac tissue or even entire hearts for transplant.

3.3 Training and education: 3D printing can be used to create realistic anatomical models for training and education purposes. In the future, medical students and trainees may use 3D printed models of the heart for practice surgeries and procedures.

3.4 Surgical planning and simulation: 3D printed models can be used to plan and simulate complex cardiac procedures before the surgery. In the future, virtual reality technology may be combined with 3D printing to create a more immersive and realistic simulation experience.

3.5 Regenerative medicine: 3D printing could be used to create scaffolds for tissue engineering and regenerative medicine. In the future, it may be possible to use 3D printing to create new cardiac tissue for patients with heart disease.

3.6 Point-of-care manufacturing: 3D printing could be used to create medical devices and implants at the point-of-care, reducing the time and cost of manufacturing and improving patient outcomes.

3.7 The Use of Artificial Intelligence In 3D Printing:

The integration of AI algorithms into 3D printing processes can help to optimize the design, manufacturing, and testing of these devices. AI can be used to analyze large amounts of data and predict the behavior of the devices under various conditions. This information can be used to improve the design, reduce manufacturing defects, and ensure the performance and safety of the devices. One example of AI in 3D printing in cardiology is the use of AI algorithms to generate designs for custom-made heart valves. The algorithms can analyze a patient specific anatomy and use this information to create a unique valve design that will provide optimal function and fit. Additionally, AI can also be used to monitor and control the 3D printing process in real-time, ensuring that the final product meets the desired specifications. This level of control can help to reduce errors, minimize waste, and improve the efficiency of the manufacturing process.

3.8 In conclusion: The future applications of 3D printing in medicine and cardiology are vast and exciting. As the technology continues to evolve, it is likely that 3D printing will become an increasingly valuable tool for diagnosing and treating cardiac diseases. With continued research and development, the potential for 3D printing to revolutionize the field of cardiology is enormous.

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Comparison Of Performances Of Machine Learning Methods: An Application On Chronic Kidney Disease

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Introduction

Chronic Kidney Disease (CKD) is a worldwide public health problem with its incidence increasing day by day, high treatment costs and negative consequences. As a result of CKD, there are not only chronic kidney failure, but also side effects such as cardiovascular diseases and weakening of kidney functions. According to already proven studies, it shows that the negative consequences of chronic kidney disease can be delayed or prevented with early diagnosis and early treatment. Unfortunately, diagnosis and treatment remain inadequate as a result of disagreements regarding the definition and classification of the disease in the progression stages of chronic kidney disease (Andrew et al., 2003).

CKD is usually caused by hypertension and diabetes. Hypertension has been cited as the cause of just over 25% of renal failure cases, and diabetes in approximately one-third of all cases. It is the most common cause of CKD in many developed countries. In addition to these diseases, although less common, inflammation, infection, kidney stones and enlarged prostate conditions can also cause CKD. In addition, CKD can occur as a hereditary disease (World Kidney Day, 2020).

In the early stages of CKD, many people are sent back before the disease can be clearly identified. In addition, an individual can lose 90% of their kidney function without showing any symptoms. CKD is very difficult to treat. With treatment, the progression of the disease can be slowed down, stopped and some serious complications can be prevented. People of all ages can get CKD. It has been determined that CKD is at a certain level in almost one out of 10 individuals. Generally, individuals of South Asian descent, African Americans, Native Americans, and Hispanics are more likely to have CKD. According to the estimation of the World Health Organization (WHO), 864,226 patients died due to CKD all over the world in 2012. On a global basis, this number is 1.5% times the total deaths. CKD ranks 14th among the leading causes of death in the world, with 12.2 people per 100,000 people.

It is foreseen that this number will continue to increase with 14 people per 100,000 people until 2030. The prevalence of CKD on a global basis and the mortality rate due to the disease are shown in figure 1 (Webster et al., 2017; World Kidney Day, 2020).

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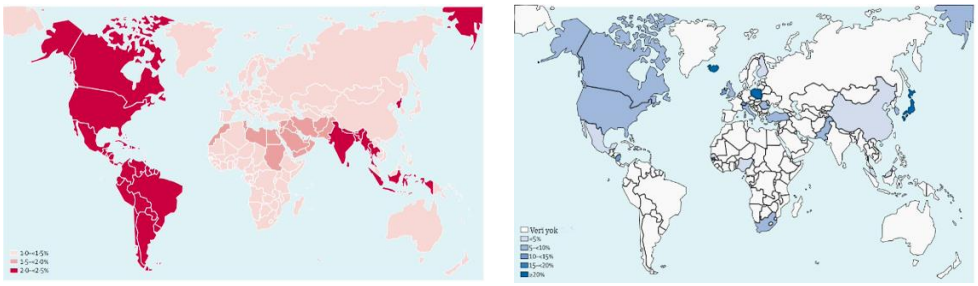

Figure 1. *Kidney disease on a universal basis a) Death rate due to kidney disease, b) Prevalence of chronic kidney disease (Webster & ark., 2017)*

The likely outcome of CKD is a highly costly treatment process in the form of dialysis or transplantation. This is end-stage renal disease requiring renal replacement. Continuation of dialysis practice with increasing momentum in middle and low-income countries, which has been between 6% and 12% in the last 20 years in developed countries, constitutes a great cost. Awareness of CKD is very low, especially in developing countries. Currently, more than 2 million people worldwide are receiving renal replacement therapy to maintain their lives, but this corresponds to only 10% of the number of patients who need treatment. The vast majority of the 2 million patients receiving treatment are treated in the USA, Germany, Brazil, Italy and Japan, which constitute 12% of the world population (Eggers, 2011; Couser et al., 2011).

In this study, machine learning algorithms were used to improve the prediction of CKD. Each algorithm was evaluated in 6 different ways, without feature selection, with correlation-based feature selection and by applying consistency criterion feature selection, in the condition that the 10-fold cross-validation technique was used or not. In this study, ZeroR, OneR, Naive Bayes (NB), Decision Tree (DT), Multilayer Perceptron (MLP), k nearest neighbor (k-NN), Logistic Regression (LR), Genetic Programming (GP), FuzzyNN and Distinctiveness Classifier (D-kNN) algorithms were used. Accuracy rate, True Positive (TP), False Positive (FP) and Roc Field analyzes were performed to evaluate and compare the performances of the algorithms. In the second part, detailed explanations of the methods and analyzes to be used in the study are given. In the third part, research findings and interpretations of the data set used are given. In the last section, the results obtained are summarized and general evaluations about the results are given.

Material and Method

Machine Learning Methods

Machine learning methods are very similar to human learning methods. People realize a serious learning by making use of their experience and experience as well as written information. Machine learning methods also make this experience and experience in humans by analyzing data sets. They make a decision based on this experience and experience. Based on these experiences and experiences, the decision-making situation can be compared to the logic of artificial intelligence. In this respect, machine learning is also considered a subset of artificial intelligence. When the training data is read, machine learning algorithms have the ability to detect data, make predictions, learn how to improve the current situation and complete tasks without waiting for commands. Transportation, automotive, sales, marketing, finance, entertainment, etc. Machine learning, which is used in many fields, is widely used for diagnosis and treatment in the medical field.

The operating steps of the machine learning process first start with the processing of the training data to the algorithm. The processed data is learned by the algorithm. It is checked by processing the test data into the system to test whether the algorithm has learned correctly. If the accuracy of the predictions is not high enough, new data is entered and the algorithm is trained repeatedly until the desired performance is achieved. In direct proportion to the data entered in machine learning algorithms, training is better provided and the accuracy rate increases. However, attention should be paid to the excessive learning and memorization of the algorithm (Saygın, 2021). The work flow chart of machine learning algorithms is shown in Figure 2 (Bilbro, 2016).

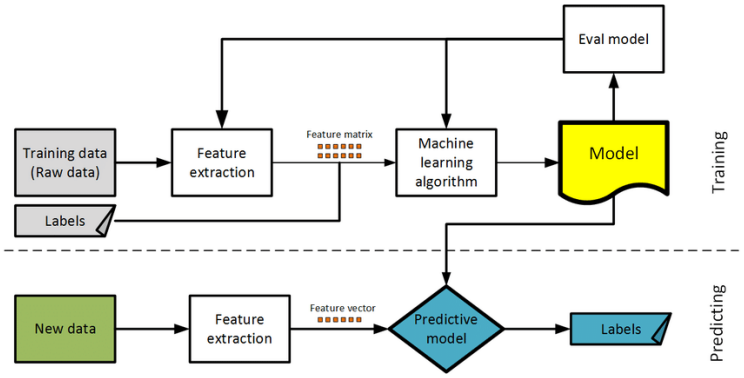

Figure 2. *Machine Learning flowchart*

ZeroR

ZeroR is one of the simplest methods of frequency-based classification algorithms. The logic of this classification method is as follows: First of all, it determines the class that is the most numerically superior to the other classes from the existing data set; After this class is determined, it converts the class of the new data to the same class with the highest number. The operation steps in the ZeroR algorithm can be shown as follows.

- Check the class of the training data in the dataset,
- Collect those belonging to the same class,
- Identify the class with the highest incidence,
- When new data comes in, include it in the class with the highest frequency.

OneR

OneR is a classification algorithm that works better than the ZeroR classification algorithm method. The working logic of this algorithm is to examine the link between the class that it wants to classify and the other classes at hand, and assign it to the class that has more similarity.

The coding for the OneR algorithm is as follows (Devasena et al., 2011)

1. For each feature A,
2. Create a rule for each VA value of the attribute as follows:
3. Add up how often each class is seen
4. Find the most frequently classified Cf class
5. Create a rule when A=VA; Class attribute value=Cf
6. End for each

7. Calculate the error rate of all rules

8. End for each

9. Select the rule with the smallest error rate.

In the OneR Algorithm, the number of training data samples that are not compatible with the binding of the attribute value in the rule gives the error rate. OneR selects the rule with the lowest error rate. If two or more rules have the same error rate, which rule is chosen randomly (Devasena et al., 2011).

Naive Bayes

The Naive Bayes Classifier is a fairly simple probabilistic classification method based on the Bayes theorem named after Thomas Bayes. It is an approach that calculates the probability that a new data belongs to any of the existing classes using sample data that is already classified. In the Naive Bayes classifier, the attributes are assumed to be independent of each other. All examples are of equal importance. The value of one property in the example does not contain information on the value of the other property. Suppose that we are working on a data set, each of which consists of n attributes and belongs to one of m classes. When it is desired to classify a new X sample whose class is unknown, the probability that the sample to be classified belongs to that class is calculated for all classes by using the first equation. The class with the highest probability value among the calculated values is accepted as the class to which the relevant sample belongs (Karakoyun & Hacıbeyoğlu, 2014).

$$P(C_i/X) = \frac{P(X/C_i)P(C_i)}{P(X)} \quad (1)$$

P(Ci|X): Probability of event Ci when X event occurs,

P(X|Ci): Probability of X event when Ci event occurs,

P(X): a priori probability of event X,

P(Ci): It is the a priori probability of the event Ci.

Since all x events have equal probability, we can simplify equation 1 as seen in equation 2.

$$P(C_i|X) = P(X|C_i)P(C_i) \quad (2)$$

Decision Tree

It is an algorithm used in decision trees, regression and classification problems. It is an algorithm used to divide a complex data set consisting of a large number of data into smaller clusters based on certain rules. While the decision tree is divided into small clusters, it simultaneously develops a related decision tree gradually (Sezer, 2008; Ezirmik, 2020). The starting cells of decision trees are called roots. Each determination is classified as yes or no according to the root condition. The determinations made are classified with the help of the nodes located under the stem cells. The more nodes, the more complex the model. At the bottom of these decision trees are leaves. Leaves form the result of the classification model (Sezer, 2008).

Advantages;

• Creating and interpreting a decision tree is simple.

• Rules can be set in an understandable way.

• Data can be processed both numerically and categorically.

• It can be used in complex and large data sets.

• It can predict discrete and continuous attribute values.

Disadvantages;

• Its success in estimating continuous values is low.

• While creating the model, if the data of the learning set in the data set is low and the number of classes is high, the success rate is not high.

• Likely to produce complex results when forming and pruning trees.

• The situation of memorizing the data set may occur.

Multilayer perceptron (MLP) algorithm

Multilayer perceptron (MLP) algorithm is a forward computation algorithm. MLP consists of a minimum of 3 layers. It consists of 3 layers, the first of which is the input layer, the second layer is the hidden layer, and finally the output layer (Demir, 2021). The number of neurons in the input layer is the same as the size of the input vector, and the amount of artificial nerve cells to be found in the output layer is determined by the state of the output vector. Although the amount of neurons in the hidden layer in the middle is at least one, it can be tested with different trials until the most beautiful and successful training is done in terms of suitability. The MLP model in Figure 3 and the multilayer perceptron equation in Equation 3.6 are given (İşeri & Arıman, 2019).

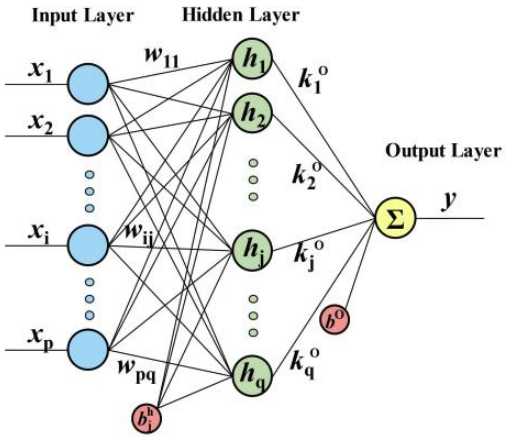

Figure 3. Multilayer sensor model (Fan et al., 2020)

$$Y_n = f_0\{b_0 + \sum_{k=1}^{h}[w_k * f_h(b_{hk} + \sum_{i=1}^{m} w_{ik}X_{ni})]\} \quad (3)$$

The variables that Equation 3 consists of are as follows:

Y : Normalized outputs

f : Function related to the transfer of the output layer

b_0 : Output layer, bias

w_k: link weight related to the output layer with the kth hidden layer

f : Function associated with transferring the hidden layer h

b_{hk}: bias terms of the kth hidden layer

w_{ik}: Connection from the second neuron in the input layer to the second neuron in the hidden layer

X_{ni}: Normalized input vector

K Nearest Neighbor (k-NN) Algorithm

The K-NN algorithm was introduced in the early 1950s and continued to be developed in the following years. This algorithm is a popular algorithm that is preferred because it can be used in both regression and classification studies. The K nearest neighbor algorithm creates a classifier for each value from the nearest neighbor cluster and assigns that value to the relevant class.

In the k-NN algorithm, k is assigned as an integer first. This assigned k value checks the k nearest neighbor values to find the class of the unknown data point. The unknown data point is estimated as the target value class with the highest frequency among k neighbors. The test data must be fine-tuned, as it can change the estimate made when the integer value of k changes. In Figure 4, the k nearest neighbor algorithm with different k values is shown on the graph below (Gençalp, 2020).

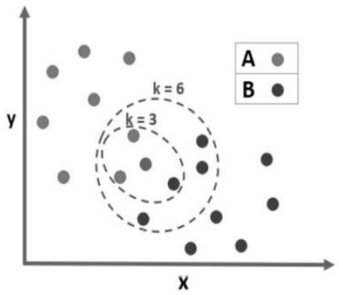

Figure 4. K nearest neighbor algorithm (Gençalp, 2020)

k-NN is one of the most used algorithms among machine learning algorithms due to its robust operation on simple, basic and noisy training data. However, because it keeps all the states in memory while making calculations about the distance, high memory needs occur when processing very large data. While this algorithm is running, first parameter k, which is the amount of nearest neighbors of a determined point, is determined. According to the determined value of the K parameter, the neighbors are determined and the classification process is applied.

Logistic Regression

Logistic Regression (LR) is an algorithm in the category of supervised machine learning algorithms that is generally used in binary (binomial) classification modeling. Binary classification modeling is used if the dependent variable only creates two types of probabilities (patient-not sick, yes-no, true-false, produced labels 0-1, etc.). If the dependent variable has three or more possibilities (hot, dry, humid, rainy, etc.), multinomial logistic regression is used, and if the dependent variable is classified by ranking more than three (bad, moderate, good, very good, etc.), the ordinal logistic regression method is used (Saygın, 2021; Kıymaz, ,2022).

Genetic Programming

Genetic programming (GP) was developed as a sub-branch of genetic algorithm method. The basis of the difference between GP and genetic algorithm is the representation of the solution structure (chromosome) and the meaning it shows. GP works to reach solutions that consist of a tree structure. Genetic programming makes it possible to find information about the content and structure of all kinds of data, without the need for the shape and size of the problem, in order to approach or solve the problem. It does this by creating a computer program that automatically activates by using a high-level value of the related problem (Alikalfa, 2013).

Fuzzy k-nearest neighbor algorithm (FuzzyNN)

The fuzzy k-nearest neighbor (FuzzyNN) algorithm is used to classify the test data in the training data set according to their similarity to a certain number of neighbors and their membership grades (fuzzy or exact) class label. In Equation 4, the calculation of the C(y) scope, where a classified object or person y belongs to a C class for FuzzyNN purposes, is shown (Jensen & Cornelis, 2008).

$$C(y) = \sum_{x \in N} R(x, y) C(x) \quad (4)$$

In Equation 3.10, N is the set of K nearest neighbors of object y, and R (x, y) is the valued similarity of x and y [0,1]. This traditional approximation calculation is shown in Equation 5.

$$R(x, y) = \frac{\|y-x\|^{-2/(m-1)}}{\sum j \in N \|y-j\|^{-2/(m-1)}} \quad (5)$$

In Equation 5 || . || It represents the Euclidean norm, and the m value is a parameter that controls the overall weight of similarity. Assuming exact classes, below is an implementation of the FuzzyNN algorithm, which assigns a test object y to the class that results in the highest degree of membership. Classification of a test model is that the complexity of this algorithm is O(|U|+ K |C|).

FuzzyNN (U, C, y, K):
U: Training data,
C: Set of decision classes,
y: Object (person) to be classified,
K: Number of nearest neighbors.

(1) N Take nearest neighbors (y, K);
(2) $\forall C \in$ C
(3) $C(y) = \sum_{x \in N} R(x, y) C(x)$
(4) Output arg max (C(y)) C∈C

Differentiability k-nearest neighbor algorithm (Dk-NN)

The purpose of the k-nearest neighbor (Discernibility k-NN) algorithm is to perform a fast classification without loss of accuracy. In terms of time complexity, dk-NN is less than other classification algorithms. This classification algorithm gives importance to the distance and distinguishability of all neighbors. This algorithm uses the distinguishability of each element, as opposed to structural density. A score is generated for all neighbors. The sample score is the distinguishability divided by the corresponding distance. Then, by taking the

average of all the scores of the class, a unique classification score is produced for each class, and the class with the highest score is selected among the classes for which the score is generated. The distinguishability calculation of each sample is made by taking the radius around each sample (the mean between that sample and all samples with similar class labels) (El-Bakry et al., 2016).

Attribute Selection

Attribution is the process of selecting the subset within a data set that can best represent that data set. It is also called feature selection and variable selection. It is the process of selecting and separating the variable with a higher function from among a number of variables in the data set. If the variables with weak correlation from the variables to be used in the data set are removed from the data set, a model with a much higher success and accuracy rate can be established.

The feature selection method first starts with the creation of the subset from the original dataset, then the created subset is evaluated. The stopping criterion comes into play to decide whether the subset under consideration is better than the current one, and the validity of the subset is checked by the validation process. Feature selection consists of these 4 main steps. The flowchart of the feature selection steps is given in Figure 5 (Dash & Lui, 1997).

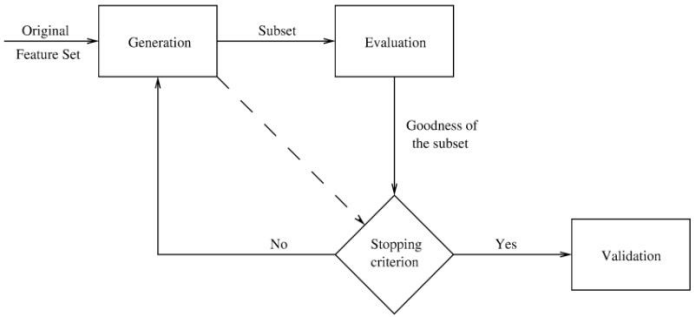

Figure 5. *Feature selection flowchart (Dash and Lui, 1997)*

Analysis Criteria for Outputs of Classification Algorithms

In machine learning methods, there are some criteria for evaluating the results after the algorithms are classified. The criteria to be used in the study TP, FP, Accuracy rate and Roc curve are detailed below.

Confusion matrix

In classification algorithms, a confusion matrix is needed to calculate the values and accuracy of the resulting model after the operations are completed. As can be seen in Table 3.1 below, it is possible to compare the difference between the estimated classification and the actual value, thanks to the confusion matrix given in 2x2 size. It is given as True:1 and False:0 in the matrix.

Table 1. *Confusion matrix (Erdursun, 2019)*

		Actual class value	
	Class	**Positive**	**Negative**
Estimated class value	**Positive**	TP	FP
	Negative	FN	TN

The terms used in the matrix given in Table 1 above are as follows;

TP (True Positive): It is the classification value and the number of values whose actual value is 1 as well.

FP (False Positive): It is the number of values that we find the classification value to be 1 but whose true value is 0.

TN (True Negative): It is the classification value and the number of values whose actual value is also 0.

FN (False Negative): It is the number of values whose classification value we find as 0 but whose true value is 1.

Accuracy Rate

The classification result consists of the ratio of the correctly estimated value to all values. Its formulation is shown in Equation 3.15.

$$Accuracy\ rate = \frac{TP+FP}{TP+TN+FP+FN} \quad (6)$$

ROC Curve

ROC (Receiver Operating Characteristics) is a performance criterion that graphically displays the relationship between TP ratio and FP ratio in relation to a classification. It shows how much the values with a value of 1 and those with a value of 0 differ from each other as a result of a classification. The ROC curve is one of the most important criteria for evaluating the performance of any classification model. It is one of the most frequently used evaluation criteria to evaluate the performance of machine learning algorithms, especially if there is imbalance in the data set. It also describes how well the model predicts. The closer the AUC (area under the curve) value on the graph is to 1, the higher the success. In other words, the AUC value provides information as a summary of the model's performance. For example, the higher the AUC value in the data set of people who are not sick and people who are sick, the higher the accuracy of distinguishing between those who are sick and those who are not sick. The graphs shown in Figure 6 provide information about the AUC value.

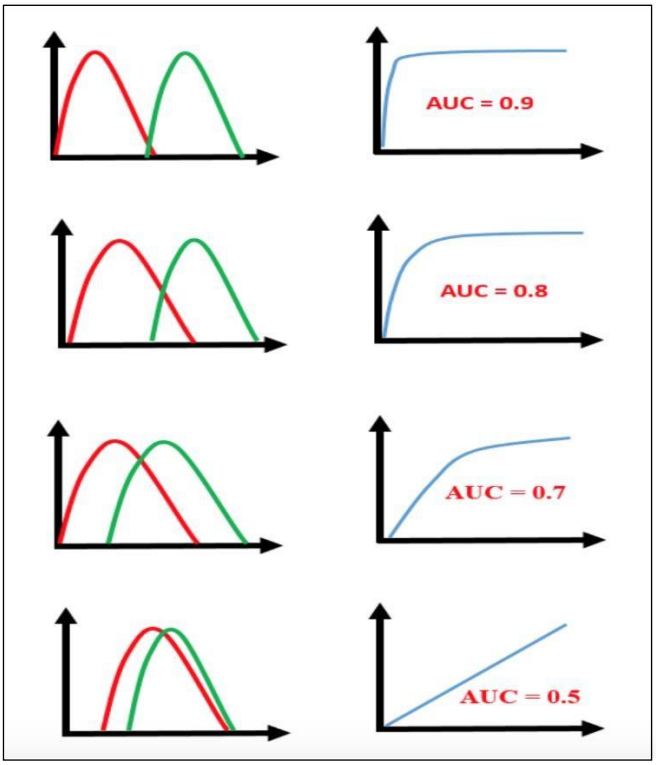

Figure 6. Graphical representation of ROC curve and AUC value (Öğündür, 2020)

As seen in Figure 6 above, 4 different AUC values are given. In the graphs on the left, the area shown in green is TP (True Positive), and the area shown in red is FP (False Positive). The area in the intersection sets are TN (True Negative) and FN (False Negative). As the red and green classes are separated from each other and the intersection set decreases, the AUC value increases and the rate of the model being a correctly predicted model increases. In the ROC curve graphs on the right, there is the FP ratio on the x-axis and the TP ratio on the y-axis. The higher the area under the curve (AUC) value, the higher the correct classification rate of the model.

K-fold cross validation method

K-fold cross validation is a model evaluation technique that divides data into k partitions and trains multiple algorithms from these k partitions. When the training and test data are separated in the learning stages of the models in the algorithms, the training data decreases, so the probability of getting erroneous results in the models of the algorithm increases. In order to avoid these errors, most of the existing data in the data set should be used during the training and validation stages of the model. For this reason, the use of k-fold cross validation method is preferred. In this method, first of all, the tuning parameter (λ) is determined and the existing data in the data set is divided into k subsets. This division happens completely randomly. With the exclusion method, the process is repeated k times, and each time a set of k subsets is used as a validation set. The remaining k-1 subsets are used as the training dataset. Thus, at all data points, the cross-validation dataset is k and the training dataset is k-1. To get the performance value of the model in Equations 3.16 and 3.17, each k. The formulation for taking the mean value of the cross validation error estimation of the section is shown (Özkan et al., 2022).

$$E_k(\lambda) = \sum i \in k\ section\ (y_i - x_i\beta^{-k}(\lambda))^2 \quad (7)$$

$$CV(\lambda) = \frac{1}{K}\sum_{k=1}^{K} E_k(\lambda) \quad (8)$$The value of λ, which minimizes the CV (λ) cross-validation error estimate value, is preferred. When a large part of the data in the data set is used in the cross-validation method for a compatible study, the rate of incorrect results is greatly reduced (Özkan et al., 2022). While using the cross-validation method, the k value is used as 10 in most of the literature studies researched. In this method, the data set at hand is divided into k pieces, one of them is used as a test set, while the other sets are used for training. This process ends when all k data are used as test sets. To give a clear example, the steps applied when k=3 is selected are shown in Figure 7.

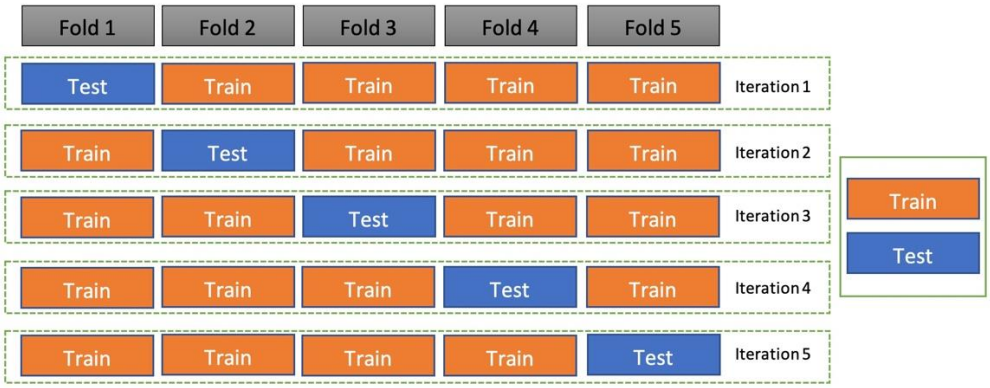

Figure 7. *Example representation of k-fold cross validation steps*

Findings and Discussion

Description of the Data Set

This study was conducted to predict CKD using machine learning methods. The data set in the study was prepared from the data collected in Apollo Hospitals in India on 03/07/2015 for approximately 2 months. The data set used in this thesis study was accessed from the UCI machine learning repository (UCI, 2022). The number of people in the dataset consisting of a total of 400 people was 250 (62%) with chronic kidney disease and 150 (38%) without. This data set consists of 25 attributes. One is the class variable. Of these variables, 14 are nominal and 11 are numerical values. The variables are introduced in Table 2.

Table 2. *Introduction of CKD dataset (UCI, 2022)*

Feature	Feature Name	Data Type	Value range
1	Age	Digital	2-90
2	Blood pressure -bp	Digital	50-80
3	specific weight -sg	Nominal	1,005, 1,010, 1,015, 1,020,1,025
4	Albumin -al	Nominal	0, 1, 2, 3, 4, 5
5	sugar-water	Nominal	0, 1, 2, 3, 4, 5
6	red blood cells-rbc	Nominal	Normal-Abnormal
7	pus cell-pc	Nominal	Normal-Abnormal
8	Pus Cell Clusters-pcc	Nominal	Yes - No
9	Bacterium-ba	Nominal	Yes - No
10	Blood sugar-bgr	Digital	22-490
11	Amount of Urea in the Blood-bu	Digital	1,5-391
12	Serum creatinine-sc	Digital	0,4-76
13	Sodium-sod	Digital	4,5-163
14	Potassium-pot	Digital	2,5-47
15	Hemoglobin-he	Digital	3,1-17,8
16	Packed Cell Volume	Digital	9-54
17	White Blood Cell Count -wc	Digital	2200-26400
18	Red Blood Cell Count -rc	Digital	2,1-8
19	Hypertension -htn	Nominal	Yes - No
20	Diabetes -dm	Nominal	Yes - No
21	Coronary Artery Disease -cad	Nominal	Yes - No
22	Appetite -appet	Nominal	Good - bad
23	Foot edema -pe	Nominal	Yes - No
24	Anemia -ane	Nominal	Yes - No
25	Class	Nominal	ckd-notckd

Model Design

In this study, while the model was designed from the data set consisting of 25 variables and 400 clinical records, ZeroR, OneR, Naive Bayes (NB), Decision Tree (KA), Multi-Layer Perceptron, k-nearest neighbor (k-NN) (k=1), k-NN (k=2), k-NN (k=3), Logistic Regression (LR), Genetic Programming (GP), FuzzyD Differential Classification (GP), FuzzyD, 12 different algorithms were used. ASUS brand computer with Intel(R) Core(TM) i7-3630QM CPU @ 2.40GHz, 16 GB RAM and Weka 3.9.5 version of WEKA package program were used to create machine learning algorithm models and take the outputs. When using the data set, two different methods were applied, without cross validation and by applying 10-fold cross validation. In addition, without feature selection, three different data sets were created using correlation-based and consistency criterion feature selection. For the performance evaluations of the algorithms, the accuracy rate, TP, FP and ROC area values were taken into consideration. Six results were obtained for each of a total of 12 different machine learning algorithms. The results obtained from this result consist of the four criteria mentioned above.

It has 25 variables without feature selection in the initial dataset. When the correlation-based feature selection method is applied to the Weka package program, the number of variables decreases to 16. The method removed 9 variables from the model that it thought were

not directly related to the disease. These nine variables consist of variables that have a high internal correlation with the independent variables but a low correlation with the class variable. Variables in the new state are shown in Table 3.

Table 3. *Correlation-based feature selection variable table*

Feature	Feature Name	Data Type	Value range
1	Blood pressure -bp	Digital	50-80
2	specific weight -sg	Nominal	1,005, 1,010, 1,015, 1,020, 1,025
3	Albumin -al	Nominal	0, 1, 2, 3, 4, 5
4	red blood cells-rbc	Nominal	Normal-Abnormal
5	Blood sugar-bgr	Digital	22-490
6	Serum creatinine-sc	Digital	0,4-76
7	Sodium-sod	Digital	4,5-163
8	Potassium-pot	Digital	2,5-47
9	Hemoglobin-he	Digital	3,1-17,8
10	Packed Cell Volume	Digital	9-54
11	White Blood Cell Count -wc	Digital	2200-26400
12	Hypertension -htn	Nominal	Yes - No
13	Diabetes -dm	Nominal	Yes - No
14	Appetite -appet	Nominal	Good - bad
15	Foot edema -pe	Nominal	Yes - No
16	Class	Nominal	Ckd - notckd

It has 25 variables without feature selection in the initial dataset. When the consistency criterion-based feature selection method is applied to the Weka package program, the number of variables decreases to 5. The method removed 20 variables from the model that it thought were not directly related to the disease. These 20 variables consist of variables with less consistency than the consistency of the attribute set. Variables in the new state are shown in Table 4.

Table 4. *Consistency criterion-based attribute selection variable table*

Feature	Feature Name	Data Type	Value range
1	specific weight -sg	Nominal	1,005, 1,010, 1,015, 1,020, 1,025
2	Albumin -al	Nominal	0, 1, 2, 3, 4, 5
3	Serum creatinine-sc	Digital	0,4-76
4	Hemoglobin-he	Digital	3,1-17,8
5	Class	Nominal	Ckd - notckd

CONCLUSION

In order to predict CKD, 6 different applications were made during the study, including 12 machine algorithm methods with cross validation and 10 fold cross validation, no feature selection, correlation based feature selection and consistency based feature selection, and their outputs were taken. These outputs were evaluated by 4 criteria, namely accuracy rate, TP rate, FP rate and ROC area. ASUS brand computer with Intel(R) Core(TM) i7-3630QM CPU @ 2.40GHz, 16 GB RAM and Weka 3.9.5 version of WEKA package program were used to create machine learning algorithm models and take the outputs. Information on the outputs of the algorithms is given in Table 5 and Table 6.

Table 5. *Accuracy rates of algorithms*

Algorithm	No Attribute Selection		Correlation Based Attribute Selection		Consistency Criteria Attribute Selection	
	Cross Validation No	Cross Validation Yes	Cross Validation No	Cross Validation Yes	Cross Validation No	Cross Validation Yes
ZeroR	62,50	62,50	62,50	62,50	62,50	62,50
OneR	92,50	92,00	92,50	92,00	92,50	92,00
Naive Bayes	94,75	95,00	96,50	97,00	96,25	95,75
Karar Ağacı	99,50	99,00	99,50	98,50	98,00	97,75
MLP	**100,00**	**99,75**	**100,00**	**99,75**	**98,50**	**96,50**
k-NN(k=1)	97,50	95,75	99,75	98,75	98,50	98,00
k-NN(k=2)	97,75	96,25	100,00	98,75	98,50	98,25
k-NN(k=3)	95,75	94,75	98,25	97,75	98,25	97,25
LR	100,00	95,75	100,00	97,50	99,75	98,25
GP	62,50	62,50	62,50	62,50	62,50	62,50
FuzzyNN	70,00	61,00	62,25	62,00	64,25	62,25
D-kNN	97,75	95,00	98,25	96,75	94,75	92,75

As a result of this study, the MLP algorithm with a very high accuracy rate of 99.75% was determined for the early diagnosis of CKD. The data used throughout the study consists of 400 clinical records. In future studies, it is aimed to carry out an application in which the test results of the individual who has undergone examinations and analyzes for early diagnosis of CKD are supported by software experts, and a computer-assisted interface is created and the probability of being sick is shown as a percentage automatically. In this way, it is aimed to provide support to the doctors in decision-making by ensuring that the probability of the patient being examined and analyzed is reduced to the system used by the doctors.

Table 6. *Performance values of algorithms*

Algorithm	No Attribute Selection						Correlation Based Attribute Selection						Consistency Criteria Attribute Selection					
	Cross Validation No			Cross Validation Yes			Cross Validation No			Cross Validation Yes			Cross Validation No			Cross Validation Yes		
	TP	FP	ROC	TP	FP	ROC	TP	FP	ROC	TP	FP	ROC	TP	FP	ROC	TP	FP	ROC
ZeroR	1,000	1,000	0,500	1,000	1,000	0,500	1,000	1,000	0,500	1,000	1,000	0,500	1,000	1,000	0,500	1,000	1,000	0,500
OneR	0,916	0,06	0,928	0,916	0,073	0,921	0,928	0,080	0,924	0,916	0,073	0,921	0,928	0,080	0,924	0,916	0,073	0,921
NB	0,916	0,000	1,000	0,92	0,000	1,000	0,944	0,000	1,000	0,952	0,000	1,000	0,940	0,000	1,000	0,932	0,000	0,999
DT	1,000	0,013	1,000	0,996	0,020	0,999	1,000	0,013	1,000	0,996	0,033	0,999	0,988	0,033	0,998	0,988	0,040	0,995
MLP	1,000	0,000	1,000	0,996	0,000	1,000	1,000	0,000	1,000	0,996	0,000	1,000	0,976	0,000	0,999	0,960	0,027	0,996
k-NN(k=1)	0,960	0,000	0,980	0,932	0,000	0,966	0,996	0,000	0,998	0,98	0,000	0,990	1,000	0,040	0,980	0,992	0,040	0,977
k-NN(k=2)	0,964	0,000	0,982	0,940	0,000	0,970	1,000	0,000	1,000	0,98	0,000	0,990	1,000	0,040	0,980	0,996	0,040	0,978
k-NN(k=3)	0,932	0,000	0,982	0,916	0,000	0,970	0,972	0,000	1,000	0,964	0,000	0,990	0,996	0,040	0,980	0,98	0,040	0,981
LR	1,000	0,000	1,000	0,952	0,033	0,988	1,000	0,000	1,000	0,968	0,013	0,998	0,996	0,000	1,000	0,984	0,020	0,995
GP	1,000	1,000	0,500	1,000	1,000	0,500	1,000	1,000	0,500	0,932	0,907	0,513	1,000	1,000	0,500	1,000	1,000	0,500
FuzzyNN	0,992	0,787	0,603	0,968	0,987	0,491	0,996	1,000	0,498	0,992	1,000	0,496	0,936	0,847	0,545	0,920	0,873	0,523
D-kNN	0,964	0,000	1,000	0,920	0,000	0,998	0,972	0,000	1,000	0,948	0,000	1,000	0,980	0,107	0,962	0,968	0,140	0,980

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Forensic Pathology Approach to Intracranial Hemorrhages

Doğuş ÖZDEMİR KARA[1]

Introduction

Intracranial hemorrhage (ICH), also known as intracerebral hemorrhage, is a catastrophic disease. The global incidence of spontaneous ICH is 24.6 per 100,000. Almost half of these deaths occur within the first 24 hours, highlighting the crucial importance of early and efficacious management in the emergency department. A meta-analysis based on a general demographic population indicated that risk factors for ICH comprised male sex, older age, and Asian origin.

Intracranial hemorrhage refers to any hemorrhage within the bones of the skull dome, including the brain parenchyma and surrounding meningeal spaces.

Intracranial hemorrhage encompasses four broad types of hemorrhage: epidural hemorrhage, subdural hemorrhage, subarachnoid hemorrhage, and intraparenchymal hemorrhage. Each type of hemorrhage results from different etiologies and clinical manifestations; the prognosis and outcomes are variable.

From the point of view of forensic pathology, it is possible to classify these hemorrhages as traumatic (non-pathological) and non-traumatic (pathological) intracranial hemorrhages according to their basic etiology. In the group classified as pathological, there is an underlying organic pathology that causes bleeding. Trauma-related brain hemorrhages are also frequently encountered in forensic pathology.

In forensic pathology, brain gross examination begins with the evaluation of the membranes surrounding the brain from the outside for hemorrhage, discoloration, and masses. Then, serial sections are made on the brain specimen, and the parenchyma is evaluated for hemorrhage, space-occupying mass, and any other possible pathology. Macroscopic examination of the brain indicates that it is surrounded by cerebral membranes called dura mater, arachnoid mater, and pia mater, respectively. The dura mater is called pachymeninges, and the arachnoid mater and pia mater together are called leptomeninges.

Epidural Hemorrhage

A dural membrane is a durable, thick fibrous membrane rich in vessels and nerves (Figure 1). The dura mater cranialis, which surrounds the brain, is divided into periosteal and meningial parts. Between these periosteal and meningial parts, dural sinuses are located (Figure 2).

The spinal part of the dura mater, which starts from the foremen magnum and ends as the filum terminale at the level of sacral 2 vertebrae (S2), is called the dura mater spinalis. The part covering the spinal cord is not in two layers like the cranial part and does not adhere to the

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vertebrae as in the skull. Thus, an epidural space is formed between the dural membrane and the vertebral column.

On macroscopic examination, the dura mater has fibrous extensions forming septations on the subdural surface (Figure 3). These fibrous septations, called falx cerebri separating the cerebral hemispheres at the level of longutidal sulcus, tentorium cerebri separating the occipital lobe and cerebellum, and falx cerebelli separating the cerebellar hemispheres, allow us to distinguish the epidural and subdural surfaces of the dural membrane sent for pathology.

The surface where we see fibrous septa is the subdural space (Figure 3), and the side where we do not see septations represents the epidural space (Figure 4). In forensic pathology, it is important to decide whether the hemorrhage is epidural or subdural in the sent dura mater sample.

Between the periosteal and meningeal parts of the dura mater, dural sinuses are present. Dural sinuses are cavities where venous blood is observed. In forensic pathology, serial incisions must be made in the superior sagittal sinus in the sent dura mater sample in terms of thrombosis (Figure 5). Unlike other organs, the venous drainage of the brain does not follow the arterial structures. The venous structures of the brain are divided into deep and superficial venous structures. Numerous veins responsible for the drainage of the cerebrum drain into the dural venous sinuses via 'bridging veins'. The venous drainage of the central nervous system and scalp drains into the dural sinuses and, from there, into the internal jugular vein (or jugularis interna). There is no valve structure in the dural sinuses.

Epidural haematomas are seen in about 2% of head trauma sufferers and account for 5% to 15% of fatal head injuries. About 85% to 95% of epidural hemomatomas have an associated overlying cranial fracture.

Epidural hematomas (Figure 6) may be of arterial or venous origin. A classical arterial epidural hematoma occurs after blunt trauma to the head, typically to the temporal region. It can also occur after a penetrating head trauma. Spinal epidural hemorrhages can also occur as a result of minor trauma or epidural anesthesia. It may also occur spontaneously in thrombocytopenias, vascular malformations, blood dyscrasias, and coagulopathies.

Typically, there is a skull fracture in which the middle meningeal artery (arteria meningia media) is damaged, potentially causing arterial hemorrhage into the epidural space. Although the middle meningeal artery is the classically identified artery, arterial bleeding from any meningeal artery can also lead to epidural hematoma. As the dura mater is tightly adherent to the skull bone, hemorrhage is self-limited in this area.

Dural hemorrhages are rare in children. Venous epidural hematoma occurs when there is a skull fracture and venous bleeding from the skull fracture fills the epidural space. Venous epidural hematomas are common in pediatric patients.

The most common symptom is a headache caused by the pressure and tension caused by the hematoma, as the dural membrane is innervated by the nervus vagon and nervus trigeminalis. As the hematoma enlarges, compression symptoms are added to neurological symptoms. Treatment requires surgical drainage of the hematoma. The mortality rate of epidural hematomas is between 5 and 50%.

The first evaluation covers airway, respiration, and circulation since patients may rapidly worsen and necessitate intubation. A comprehensive neurological examination assists in the identification of neurological impairments. Cushing's reaction (hypertension, bradycardia, and bradyypnea) may be observed as intracranial pressure increases. A non-contrast emergency CT

head is a valid imaging option due to its good sensitivity and selectivity to detect substantial epidural hematomas.

Subdural Hemorrhage

The frequency of subdural hemomatoma (Figure 7) is reported to be between 5% and 25% of patients with major brain trauma. The yearly frequency is one to five cases per 100,000 inhabitants, with a male-to-female ratio of 2:1. The frequency of subdural haematomas rises during life.

It is thought to occur secondary to trauma in the bridging veins opening to the dural sinuses. Especially in the elderly, there is a tendency to subdural hemorrhage due to stretching of these veins because of shrinking brain tissue due to atrophy, and similarly, in infants, venous structures are more fragile.

Additionally, abuse-related head trauma (AHT) is related to both direct and indirect spinal injuries, such as spinal subdural hemorrhage (SSH). The documentation of proof of spinal injury, such as spinal SDH, indicates that it may have an active role in the identification and subsequent management of the child with AHT and his or her siblings.

Intracranial aneurysm rupture is a rare and under-recognized cause of SDH that can occur without concurrent subarachnoid hemorrhage. The mechanism of aneurysmal SDH is controversial, but understanding the anatomical microenvironment of the aneurysm offers insight and explains the aneurysm characteristics that predispose to subdural compartment rupture.

In the so-called chronic subdural hematoma, which is frequently encountered in forensic pathology practice, blood, degenerated blood elements, and fluid accumulation become encapsulated in the subdural space. In this area, re-bleeding may occur due to fragile, newly formed capillaries in the granulation tissue.

The documentation of subdural hematomas, acute hemorrhage (Figure 8), and chronic (organized, Figure 8) hemorrhage is performed microscopically and is an important part of forensic pathology practice (Table 1).

Treatment of subdural hematoma begins with ensuring adequate airway, breathing, and circulation, which are the basis of resuscitation. Intubation should be considered if the patient's Glasgow score (GCS) is worsening or if the GCS is 8 or lower. Since subdural hematoma may require urgent surgical intervention, the case should be evaluated by neurosurgery. Because the definitive treatment for subdural hematomas is surgery, However, in some cases, depending on the size and location of the hematoma, some subdural hematomas may be followed by spontaneous resorption.

Subarachnoidal Hemorrhage

The arachnoid membrane is a thin connective tissue membrane, and the potential area between it and the pia mater is called the subarachnoidal space. The arachnoidal membrane forms finger-like projections called granulomas towards the subarachnoidal space. There are also spider web-like extensions from the arachnoid membrane towards the pia mater. These extensions are called arachnoidal trabeculae.

The subarachnoidal space contains the main vascular structures of the brain (anterior cerebral artery, posterior cerebral artery, medial cerebral artery and its branches, and vertebrobasilar arteries), nerves (cranial nerves), and cerebrospinal fluid (cerebrospinal fluid,

CSF). In the subarachnoidal space, the relatively large areas where CSF is pooled are called 'cisterna'. Within these cisterns, vital anatomical structures are present.

The cisterna magna is the largest and is filled with two lateral and one median aperture in the fourth ventricle. The nervus vagus, vertebral arteries, and glossopharyngeal nerve pass through it.

The pontine cistern is located anterior to the pons. The basilar artery, superior cerebellar artery, anterior inferior cerebellar artery, and nervus abducens are located here.

The chiasmatic cistern is located above the sella tursica, below the hypothalamus. The optic chiasm and pituitary are located in the stalk.

The interpeduncular cistern is located in the interpeduncular fossa, surrounded by cerebral peduncles. It contains the bifurcation region of the basilar artery, posterior cerebral artery, superior cerebellar artery, and its bridge, mammary bodies, and oculomotor nerve.

CSF is produced by the filtration of plasma from blood in the choroid plexuses located in the ventricles. Choroid plexuses are vascular bundles lined with ependymal cells. CSF carries nutrients and ions to the brain parenchyma and removes toxins and waste from the brain parenchyma. At the same time, CSF also acts as a layer of fluid that surrounds the brain parenchyma from the outside and provides suspension against various traumas. After circulating the whole brain, it drains from the subarachnoidal space to the dural sinuses through arachnoid granulomas and joins the venous circulation.

Subarachnoid hemorrhage (SAH) causes nearly 5% of all strokes. In people over 35 years of age, the incidence is between 2 and 25 per 100,000. This incidence increases slowly with age and may be slightly more common in women than in men (female-to-male ratio of 1.15:1). Identified risk factors associated with SAH include current and former smoking history, hypertension, and excessive drinking.

Subarachnoidal hemorrhages are mostly caused by rupture of intracranial aneurysms, also known as BERRY or saccular aneurysms (Figure 11). These aneurysms occur at the branching points of the main cerebral vessels and the thinning points between the internal elastica and media (Figure 12).

It is seen especially in the first bifurcation of the MCA (middle cerebral artery) in the Willis polygon. The defect in the vessel wall is present from childhood and becomes saccular with the effect of blood pressure with age.

Intracranial aneurysms are common in women, in patients with aortic coarctation, in polycystic kidney disease, and in patients with collagen tissue disease. The genetic inheritance that predisposes to aneurysms is mediated by genes encoding collagen in the vasculature.

A recent study revealed that in a population free of comorbidities, the prevalence of unruptured intracranial aneurysms was 3.2%. This suggests that only a small percentage of these aneurysms rupture and cause SAH. Studies have shown that the risk of rupture of aneurysms is increased in those with a history of SAH, those over 60 years of age, and the female gender. In addition, the risk is higher in aneurysms larger than 10 mm and those in the posterior circulation.

Since the cranial main arteries run in the subarachnoidal space, subarachnoidal hemorrhage is the most common cause (Figure 13, Figure 14, Figure 15). If the blood flow is towards the brain parenchyma, intraparenchymal and/or intraventricular hemorrhage may also occur.

Large aneurysms may also cause symptoms by compressing the surrounding neurological structures. Determining the cause of non-traumatic subarachnoid hemorrhage will help direct further treatment.

Common tests include a CT angiogram (CTA) of the head and neck, magnetic resonance angiography (MRA) of the head and neck, or diagnostic cerebral angiogram of the head and neck performed in an emergency to look for an aneurysm, AVM, or other sources of subarachnoid hemorrhage.

In forensic pathology, subarachnoidal hemorrhages are also evaluated based on the presence of inflammatory cells and other repair cells at the site of hemorrhage. A detailed description is given in Table 2.

Intraparenchymal Hemorrhage

Intraparenchymal (IPC) hemorrhage accounts for 10% to 20% of all strokes. The incidence of intraparenchymal hemorrhage increases with increasing age in people aged 55 years and older. There is some controversy about gender differences, but there may be a slight male predominance.

Pathological intraparenchymal hemorrhages are often caused by the rupture of small vascular structures secondary to hypertension. Small vascular structures penetrating the parenchyma lose their elasticity under the impact of hypertension. Over time, chronic stress on the vessel walls leads to fragmentation, degeneration, and the eventual rupture of small penetrating vessels within the brain parenchyma. This entity is a hypertensive vasculopathy known as lipohyalinosis, which causes microscopic degenerative changes in the walls of small and medium-sized penetrating vessels. Such hemorrhages are mostly seen in the basal ganglia (Figure 16) and thalamus. However, it may also occur in the cerebellum, pons, and subcortical cerebrum.

The 30-day mortality rate for IPH varies between 30% and 55%, and half of the mortalities occur within the first 48 hours.

Microscopically, ischemia and edema are seen in the tissue surrounding the hemorrhage due to compression (Figure 17). Perihematomal edema develops within the first 3 hours after the onset of symptoms and peaks between 10 and 20 days. Subsequently, blood and plasma products mediate secondary damage processes such as the inflammatory response, activation of the coagulation cascade, and iron deposition from hemoglobin degradation. Hemorrhage and edema increase intracranial pressure and cause neuronal deficits and symptoms specific to the site of hemorrhage.

The vascular system providing brain nutrition is divided into two parts: the anterior and posterior circulation. Anterior, posterior, and medial cerebral arteries form the anterior circulation, while vertebrobasilar arteries form the posterior circulation.

The brain receives 15% of the cardiac output. Neuronal cells can only use glucose as energy. Neuronal cells can only use glucose as an energy source (no glycogen is stored in the cells). A continuous supply of oxygen and glucose to the cells is essential. Severe damage can occur within 5 minutes in the absence of oxygen and within 15 minutes in hypoglycemia. Therefore, even a short interruption of cerebral blood flow results in serious damage.

The presence of red neurons and edema in the ischaemia areas indicates the occurrence of hypoxic ischaemia during the acute period. Red neurons are characterized by hypereosinophilic nuclei. Upon macroscopic examination, these old ischaemic areas are observed to be cystic and thinned, as shown in Figure 18. These findings suggest that even a

brief interruption of cerebral blood flow can lead to significant damage, emphasizing the importance of maintaining adequate oxygen supply to the brain. These findings are consistent with the effects of prolonged oxygen deprivation and reduced blood flow to the brain. The presence of cystic and thinned areas suggests that these ischemic areas have undergone tissue necrosis and atrophy over time.

Table 1-Microscopic evaluation of subdural hemorrhages

24 hours before	-Regular erythrocytes -Some fibrin between the dura and hemorrhage (hematoma)
24-48 hours	-Increase in fibrin deposition -The arrival of neutrophils at the hemorrhage site -Proliferation of fibroblasts at the interface of the dura and hematoma
48-72 hours	-Increase in fibrin, neutrophil and fibroblast density -Endothelial proliferation
3-5 days	-The appearance of macrophages -Early destruction of erythrocytes -3rd day: At the end of the day, pseudomembrane is formed and fibroblastic layer thickness reaches 3-4 cell thickness -5th day: Pseudomembrane thickness reaches 7 cell thickness at the end of the day
Until 14 days	-Hemosiderin-laden macrophages are observed -Pseudomembrane thickness increases up to 2 times the thickness of the original dura -Giant new capillary vessel formations are observed
1 month	-The thickness of the pseudomembrane is approximately the thickness of the dural membrane -Accumulation of collagen occurs -Arterial proliferation is observed
6 months	-Infrequently hemosiderin-laden macrophages are observed -Pseudomembrane merges with dural membrane -Rare blood vessels are observed
1 year time	-A fused pseudomembrane that is difficult to distinguish from the original dural membrane - Hemosiderin-laden macrophages are still present

Table 2: Microscopic evaluation of subarachnoidal haemorrhages

1 hour	-Fresh hemorrhage is present in the subarachnoidal space
1-4 hours	-Neutrophils are seen occasionally -Occasionally lytic erythrocytes are present -Etravasation of erythrocytes through the Virchow- Robin gap
12-24 hours	-Hemosiderin and fibrin are seen in the subarachnoidal space -Increase in the number of lymphocytes and macrophages
24-48 hours	-Increase in neutrophils and macrophages -Significant hemosiderin deposition
3th day	-Neutrophil infiltration is at maximum level
5th day	-Pooling of erythrocytes -Increased lymphocytes -Dense fibrin deposition separates erythrocytes into islands -Early collagen deposition
1 week	-Hemosiderin-laden macrophages are present -Neutrophils are no longer visible -Few intact erythrocytes remain
10 days	-Fibrosis -Breakdown of erythrocytes is almost complete
2 weeks	-Some erythrocyte destruction continues -Hematidine macrophages -Increase in additional fibrin collagen and phagocytosis
4 weeks	-Recurrent bleeding -Meningial reactive changes -Various amounts of mixed inflammatory cells
1 month	-Sometimes macrophages and haemosiderin can be seen for years

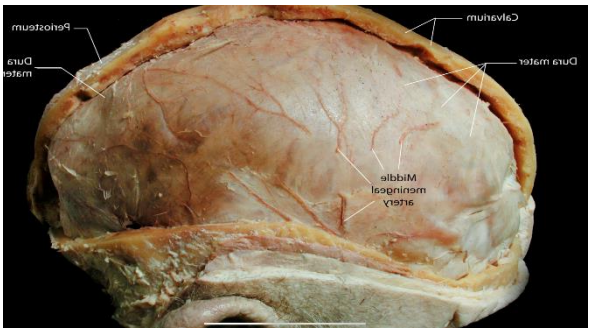

Figure 1: Duramater is a thick fibrous meningeal membrane located between the scalp and cranium.

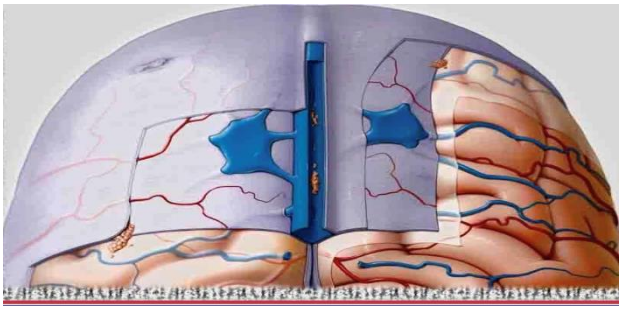

Figure 2: Between the periosteal and meningial duramater, the dural sinuses are located.

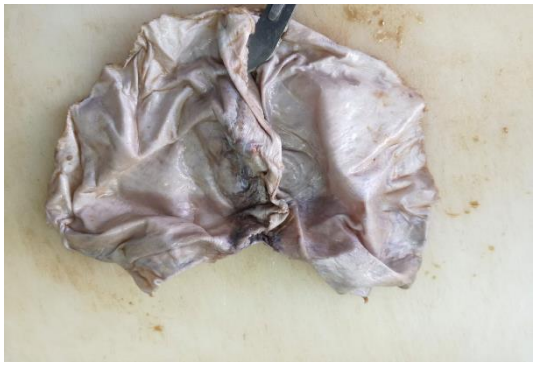

Figure 3: Fibrous septation on the subdural surface of the dural membrane.

Figure 4: There is no fibrous septation in the epidural space.

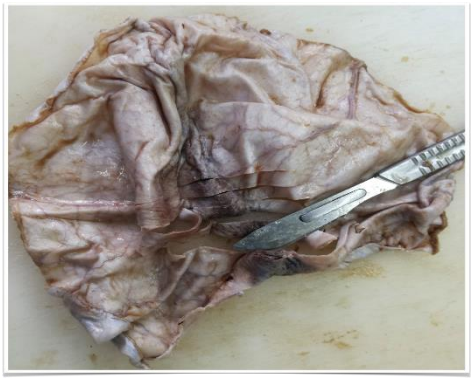

Figure 5: Serial sections must be made in the superior sagittal sinus for thrombosis.

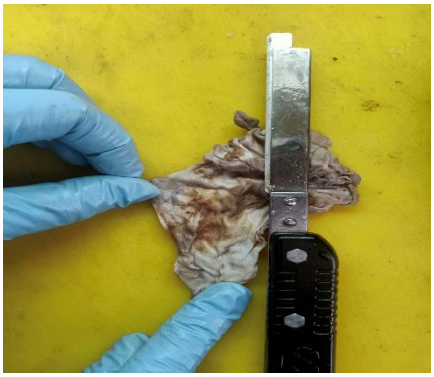

Figure 6: Macroscopic haemorrhage on the epidural surface.

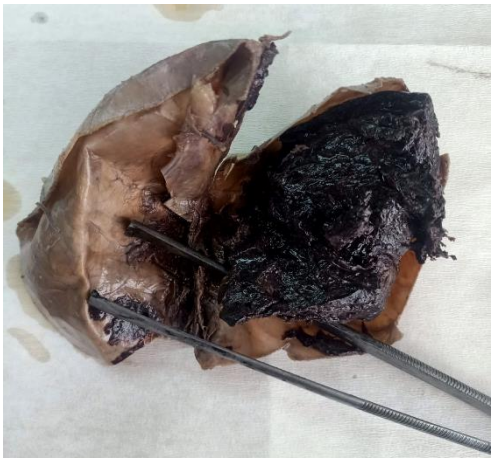

Figure 7: Subdural haematoma forming a thick coating macroscopically.

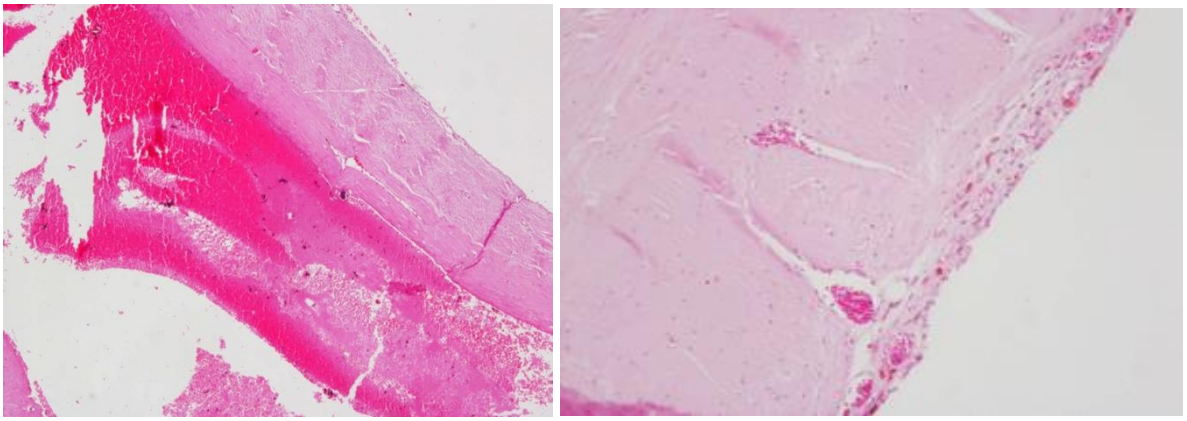

Figure 8: Microscopic examples of acute subdural haemorrhage (on the top) and chronic (organised) subdural haemorrhage (at the buttom)

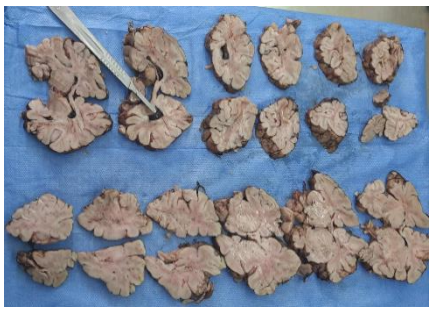

Figure 9: Choroid plexus vascular structures in the lateral ventricle macroscopically

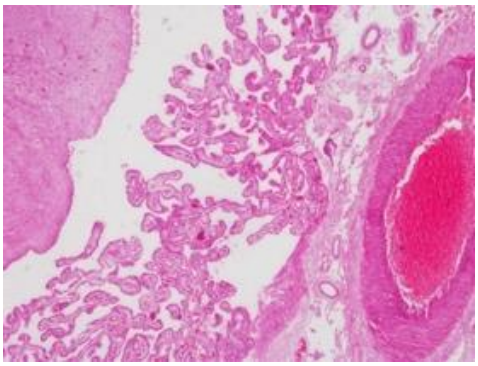

Figure 10: Microscopic view of the choroid plexus.

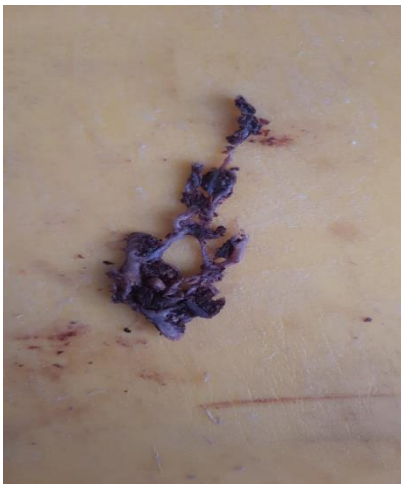

Figure 11: Macroscopically observed aneurysmatic dilatation in the right posterior cerebral artery

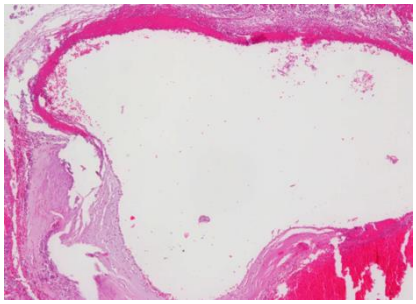

Figure 12: Microscopy of aneurysmatic dilatation and rupture of the main cranial vascular structure

Figure 13: Macroscopic subarachnodal haemorrhage in both lobes of the cerebellum

Figure 14: Macroscopic subarachnoidal haemorrhage in a piece sent from the cerebrum.

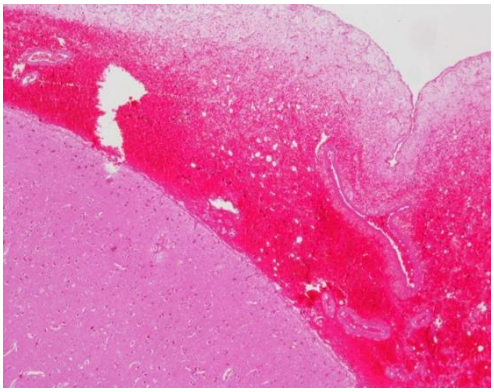

Figure 15: Microscopic section prepared from the cortex showing diffuse haemorrhage in the subarachnoidal space.

Figure 16: Macroscopic appearance of haemorrhage in the basal ganglia secondary to classical hypertension.

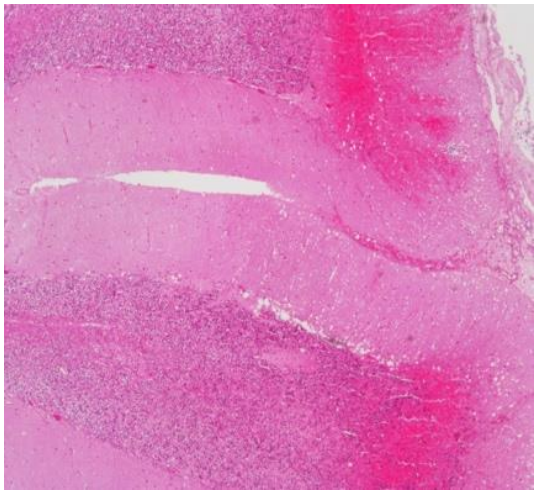

Figure 17: In microscopic sections of the cerebellum, edema characterised by fresh haemorrhage and vasuolised appearance in the surrounding parenchyma.

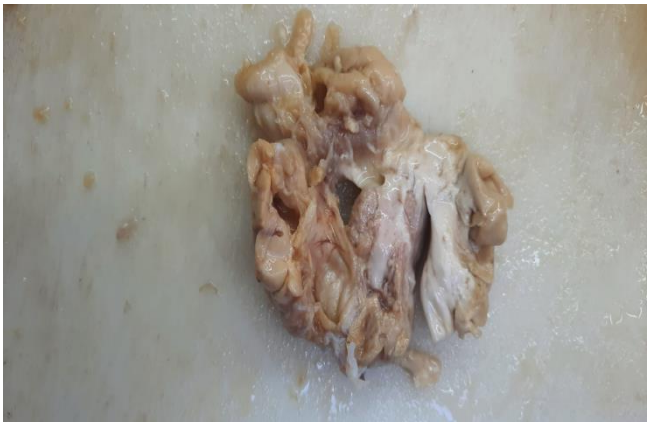

Figure 18: Old ischaemic areas are found to be cystic and thinned on gross examination

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Applications of Artificial Intelligence in the Perinatal Period

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Tugba AKCAOGLU[2]

Introduction

The practice of artificial intelligence (AI) technology in obstetrics includes monitoring maternal and fetal health during pregnancy, early disease diagnosis and treatment in the perinatal period, and postpartum follow-up. During the perinatal period, the primary goal is to improve maternal and fetal health, reducing morbidity and mortality rates. AI-based systems are primarily focused on predicting pregnancy complications. This chapter highlights the significance of these new technologies during the perinatal period.

Artificial Intelligence Applications

In recent decades, the use of artificial intelligence (AI) has significantly expanded. AI has been applied in Obstetrics in nine areas, including general pregnancy risk assessment, prenatal diagnosis, pregnancy-related hypertension disorders, fetal growth, stillbirth, gestational diabetes, preterm deliveries, delivery route, and other related fields. AI-based solutions appear promising for predicting pregnancy disorders and complications when searching databases such as Pubmed/MEDLINE, Web of Science, Cochrane Library, EMBASE, and Google Scholar. Artificial neuronal network (ANN) methods are the most accurate way to assess medical conditions, with an average of 80-90% accuracy based on current data.(Feduniw et al., 2022).

Advanced methods for predicting, diagnosing, detecting early, and monitoring perinatal health are emerging. Many experts use machine learning (ML) as an artificial intelligence technique to predict childbirth-related issues. These may include preterm birth, birth weight, preeclampsia, mortality, hypertensive disorders, and postpartum depression. ML relies on mathematical, statistical, and computational science methods to analyze multiple variables at the same time.(Mennickent et al., 2023).

Real-time electronic health recording and predictive modeling can lead to better fetal monitoring and improved outcomes for women with gestational diabetes. AI-based applications have great potential to enhance prenatal diagnosis of congenital disabilities and assisted reproductive technology. (Feduniw et al., 2022).

Pregnancy Diseases and Complications

Proper care during pregnancy, childbirth, and postpartum is crucial to ensure the health of both mother and baby. Predictive models can assist in reducing mortality rates. The amount of data routinely recorded in neonatal care has significantly increased. This vast quantity and range of information can be utilized by artificial intelligence to aid in decision-making processes and personalized care. These current approaches have great potential to be an efficient

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tool. It is crucial to comprehend the constraints and establish standardized care. (Kwok et al., 2022)

Wearable technologies are becoming increasingly popular for monitoring the cardiorespiratory health of newborns (Sitaula et al., 2023). The most common wearables are clothing (39%), chest/abdominal belts (25%), and adhesive patches (15%). These devices use sensors to measure physiological information such as electrocardiogram, breathing, oxygen saturation, and photoplethysmography. (Grooby et al., 2023).

One of the top concerns for obstetrics and gynecology specialists is gestational diabetes mellitus (GDM). It is crucial to accurately estimate the birth weight of neonates from women with GDM. Timely antenatal intervention, based on accurate fetal weight estimation, can help lower the risks of future diseases. This is why an artificial neural network is being discussed. (Zhou et al., 2022).

Placenta and Its Related Pathology

Although limited data has been collected to investigate the placenta and related pathology, digital imaging is increasingly applied. Research on digital imaging of the placenta often involves techniques like immunohistochemistry, confocal microscopy, 3D reconstruction, and deep learning algorithms. Clinical and radiology correlation can potentially improve fetal and maternal health care, including targeted therapy selection for high-risk pregnancies. (Marletta et al., 2023).

Placenta acreata spectrum with cervical implantation is a severe condition. Early identification using a risk warning model is crucial for a proper treatment plan. AI models are used in estimating such cases. (Xin et al., 2022) The placenta is a crucial organ to be explored during pregnancy. A study was conducted to investigate the molecular mechanism of the peroxizome-proliferator activator receptor (PPAR) signaling pathway in the placenta during in vitro fertilization – embriyo transfer (IVF-ET) in the first trimester. The study found that the IVF-ET treatment in the first trimester impacted the gene expressions and functions in the PPAR signaling pathway in the placenta. This conclusion suggests that this could be a potential mechanism for developing various adverse outcomes during the perinatal period. (Zhao et al., 2019)

Preeclampsia

Preeclampsia (PE) significantly contributes to perinatal health complications and death. Despite extensive research, assessing PE risk during early pregnancy is not yet a standard practice. Using AI and ML to predict the risk of PE and its subtypes will aid in creating clinically relevant risk prediction algorithms, allowing for timely intervention and developing new treatment strategies.(Hedley et al., 2023).

A broad range of clinical data is considered when evaluating a patient, including medical history, physical symptoms, and laboratory parameters. Early-detection machine-learning models can use novel biomarkers, such as soluble fms-like tyrosine kinase-1 and placental growth factor. Schmidt LJ et al. presented two distinct ML models, namely a gradient-boosted tree and a random forest classifier, to enhance predictions of adverse outcomes in pregnant women with a high risk of PE. Their findings validate the effectiveness of machine learning techniques in this regard. Moreover, they developed an automated system that eliminates the need for manual tuning or adjustments. (Schmidt et al., 2022).

Prediction of Perinatal Mortality

A study conducted by Bogale et al. aimed to predict perinatal mortality by analyzing mothers' health status and insurance coverage. They utilized homogeneous ensemble machine learning methods that involved developing a predictive model using cat boost, random forest, and gradient boosting algorithms. The model was then assessed using objective (accuracy, precision, recall, receiving operating characteristic, F1_score) and subjective (domain expert) evaluation techniques. The researchers identified the risk factors of perinatal mortality by analyzing feature importance and extracting relevant rules from the best-performing model. The most significant risk factors of perinatal mortality were community-based health insurance, mother's educational level, region and place of residence, age, wealth status, birth interval, preterm, smoking cigarette, anemia level, hemoglobin level, and marital status. (Bogale et al., 2022). In a systematic review of literature on predicting mortality during and after pregnancy, the top five features commonly used to train models were birth weight, gestational age, sex of the child, Apgar score, and mother's age. These models are expected to enhance the quality of life for mothers. (Silva Rocha et al., 2022)

Latest Investigations

Asymptomatic pregnant women with short cervical length undergo evaluation of perinatal outcome using amniotic fluid metabolomics and proteomics, sonographic, clinical, and demographic factors, including deep learning and other ML techniques.(Bahado et al., 2019)

Assessing a baby's overall well-being and neurological development can be aided by heart rate variability (HRV). HRV can serve as a monitoring system for neonatal intensive care units. Additionally, recent studies have focused on creating models that can effectively monitor and predict the clinical conditions of infants. (Chiera M et al., 2020)

A machine learning algorithm estimates gestational age at birth by processing the light scatter signal acquired on the newborn's skin. (Vitral et al., 2023).

A deep learning-based method was used to predict cerebral palsy at 13 hospitals in Belgium, India, Norway, and the US from 2001 to 2018. The method showed high accuracy during external validation. (Groos et al., 2022)

Using a model that combines clinical perinatal factors and neonatal difusion tension imaging measures of white matter microstructure, impaired language development resulting from preterm birth can be predicted and scaled across centers. (Valavani et. al, 2021).

Important Remarks

Radiographers in the UK are planning to research using AI to address critical clinical challenges. However, integrating AI into healthcare ecosystems to benefit patients and service users requires guidance. (Malamateniou et al., 2021)

When caring for newborns in the delivery room, it is essential to consider the latest technological advancements. These include respiratory function monitors, electoral impedance tomography, video laryngoscopy, augmented reality, video recording, eye tracking, contactless monitoring, and AI. These tools allow healthcare providers to provide the best possible care for newborns. (Batey et al., 2022)

Conclusion

Medical fields have seen significant growth in the use of artificial intelligence. Pregnancy-related diseases and complications are now utilizing AI applications. These

approaches aid in diagnosis, treatment, and understanding of perinatal changes. Adult sensor technologies have been implemented and tested, but not yet explored in newborns. Challenges include working with different data types, analyzing large amounts of information, developing emerging technologies, and conducting translational studies. Machine learning systems will continue to grow in managing the perinatal period in obstetrics and gynecology.

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Co_2 Laser Interventions in the Treatment of Stress Urinary Incontinence

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Introduction

The most prevalent form of urinary incontinence in women is stress urinary incontinence (SUI) (Ranjbar & et al., 2022). Around 35% of women in their reproductive years are affected by it (Gaspar & et al., 2022). Four primary mechanisms explain the causes of SUI, including increased abdominal pressure and chronic ischemia of pelvic floor muscles, endocrine changes, pelvic structural damages, and inflammatory and consumptive states. This common condition can be treated through conservative measures or surgery (Song & et al., 2022). Effective nonsurgical treatments are increasingly preferred over surgery due to lower risks (González-Isaza & et al., 2022). Laser therapy can help reconstruct collagen for vaginal and pelvic floor support (Montera & et al., 2022). Recent studies suggest that laser therapy can improve or even cure SUI (Alexander & et al., 2022). In this document, we provide a summary of the most recent studies in a single chapter.

A review conducted in 2022 analyzed 256 relevant records in literature databases and registers, as well as 25 additional searches involving 431 patients. The study found that using CO2-laser and Erbium: YAG-laser therapy for SUI treatment is a fast, easy-to-understand, and well-tolerated procedure. However, the long-term effects of this treatment have yet to be entirely determined (Ranjbar & et al., 2022).

A study involved women aged 18 to 80 with objective and subjective SUI. The trial was conducted across multiple centers and was participant-blinded. Fifty-two participants received laser treatment, and 49 received sham treatment. After three months, there was no significant difference between the two groups in terms of subjective SUI or objective SUI. The study also found that the two groups had similar patient-reported outcomes and health-related quality of life. However, vaginal bleeding occurred in three participants who received laser treatment and one who received sham treatment. Pain levels during treatment were comparable between the two groups. The study found no significant improvement in SUI with CO2 vaginal laser therapy compared to sham treatment (Alexander & et al., 2022).

A new study evaluated the safety and effectiveness of a non-ablative Er: YAG laser treatment for SUI in forty-three female patients. Each patient underwent three IncontiLase® procedures, and the success of the laser treatment was measured using various tests and questionnaires at multiple follow-up appointments. Patients were asked about any discomfort experienced during the treatment and adverse effects after the procedures. The results showed a significant improvement in all outcome measures throughout the clinical trial. However, after eighteen months, the effect began to fade, but this was resolved by single-session maintenance treatments every six months. No serious adverse effects were reported during the study, and

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any reported side effects were mild and temporary. The non-ablative Er: YAG laser treatment significantly improved SUI symptoms, with high success rates and patient satisfaction maintained through regular maintenance treatments (Gaspar & et al., 2022).

A study conducted by Long et al. aimed to evaluate the effectiveness of Pixel CO2 laser treatment in treating female SUI. The study included 25 women with SUI who were scheduled to undergo vaginal Pixel CO2 Laser (FemiLift™, Alma Lasers, Israel) treatment. Subjects were assessed at baseline and six months post-treatment using three-dimensional perineal ultrasound and validated questionnaires. The results showed that after three sessions of laser treatment, SUI symptoms significantly improved according to validated questionnaires. Perineal sonography revealed a significant decrease in bladder neck mobility and the middle urethral area during resting and straining. No permanent adverse events were reported. Therefore, Pixel CO2 Laser is an effective and safe treatment for mild to moderate SUI symptoms (Long & et al., 2022).

Another study conducted by Lauterbach et al. aimed to assess the effectiveness and safety of a single carbon dioxide (CO2) laser maintenance treatment in women who had previously undergone laser treatments for SUI but experienced a decline in the treatment's effect. The study included women aged 40-70 who had shown significant temporary improvement in SUI symptoms following CO2 laser treatments. The participants were randomly divided into two groups - the treatment group and the sham treatment control group. The researchers collected data on cough test results, 1-hour pad weights, and scores on the Urogenital Distress Inventory (UDI6), the International Consultation of Incontinence Questionnaire (ICIQ-UI), and the Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQ-12) at baseline, three months, and six months. One hundred eighty-three women were screened, of which 131 were included in the final analysis. The groups showed similar demographic characteristics and baseline measures in the outcome tests. At three months post-treatment, the study group demonstrated statistically significant improvements compared to the control group. However, the values at six months post-treatment were similar to those at baseline (Lauterbach & et al., 2022).

Seki and colleagues conducted a study to assess the effectiveness of using CO2 laser and radiofrequency in treating SUI. The study involved 139 women with SUI complaints who were randomly divided into three groups: the radiofrequency, laser, and control groups. The patients attended outpatient treatment sessions every three months for a year. The effectiveness of the treatments was evaluated using a Likert scale and objective findings from negative stress tests, voiding diaries, and pad tests. The study revealed that the CO2 laser and radiofrequency groups had similar results, which were better than those of the control group (Seki & et al., 2022).

A study by Montera et al. aimed to assess the effectiveness of using a CO2 intravaginal laser for treating SUI in patients waiting for anti-incontinence surgery (TVT-O). The study involved 52 patients divided into two groups based on whether they had atrophy. The results showed that the CO2 laser treatment improved all the SUI parameters for both groups, but the improvements were more significant for the atrophy group (Montera & et al., 2022).

In another study conducted by Gao et al., it was shown that vaginal fractional carbon dioxide (CO2) laser treatment could lead to remodeling vaginal biomechanical and physiological properties for patients with SUI. The study included 26 patients between 2019 and 2020 who received two sessions of FemTouch vaginal fractional CO2 laser at a one-month interval. The results indicated that this treatment can enhance vaginal tightening and improve pelvic floor structures, thus restoring vaginal biomechanical and physiological properties (Gao & et al., 2023).

In order to determine the effectiveness and safety of vaginal energy-based treatments for female SUI, Zhang C and their team searched various databases, including PubMed, EMBASE, Web of Science, and Scopus up until September 2022. They identified 6 RCTs that compared energy-based therapies with placebo interventions, and 577 patients were included in the study. The primary outcome assessed was the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) score, while the secondary outcomes included the cure rate and the 1-hour pad test. Upon conducting a meta-analysis, it was found that there was no significant difference in the efficacy of energy-based therapy compared to placebo intervention (Zhang & et al., 2023).

When examining the cause, we find that a weak connection between the urethra and bladder due to childbirth and decreased estrogen during menopause can result in SUI. Ruffalo et al. reported a significant improvement in symptoms, as measured by the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-UI-SF), six months after vaginal laser therapy. The cure rate ranged from 21% to 38% (Ruffolo & et al., 2022).

A prospective study was conducted on 59 women to evaluate the effectiveness of fractional/pixel CO2 laser treatment when applied intravaginally. The treatment was administered in 3 sessions, spaced 4-6 weeks apart, and evaluations were conducted at 3, 6, and 12 months. No significant adverse effects were observed. The authors concluded that the treatment was successful and recommended maintenance treatments at intervals of 6-12 months for these patients (Nalewczynska & et al., 2022).

There is a growing interest in noninvasive treatment for female SUI, leading to studies on the effectiveness of laser treatment. Research from PubMed, Medline, the Cochrane Library, and Web of Science was analyzed, showing improvement rates ranging from 62% to 78%. The studies also found no significant adverse events but minor side effects such as a sensation of warmth, increased vaginal discharge, and temporary urge urinary incontinence (Conté C & et al., 2017).

In a study conducted using the MonaLisa T Fractional CO2 laser system by DEKA, transvaginal treatments were given to 58 women every 4-6 weeks for three sessions. After completion of the treatment, 82% of participants reported an improvement in their SUI symptoms, ranging from mild to no symptoms, with significance. However, the treatment effect slightly decreased during follow-up assessments. At 12-24 months, 71% of participants still reported ongoing improvement in their SUI symptoms with statistical significance (Behnia-Willison F & et al., 2019).

A new treatment method called energy-based treatments shows promise in improving symptoms of SUI. A two-center study was conducted on 85 women with symptoms of SUI as determined by the cough stress test to evaluate its effectiveness. It validated International Consultation on Incontinence Questionnaire-Urinary Incontinence Short Form (ICIQ-UI-SF). The women were treated with Pixel-CO2 laser intravaginally in two sessions, one month apart. There were no reported adverse events or significant pain. The study showed the efficacy and safety of this treatment (Franić D & et al., 2020).

In a study by Dabaja et al., the safety and effectiveness of CO2 laser therapy for SUI in women was examined. A total of 33 women, with a mean age of 43 years, were included in the study after being evaluated by a urologist/urogynecologist and diagnosed with SUI through urodynamic testing. The women underwent three laser therapy treatments and were assessed at 1, 3, and 6 months. Despite the small sample size, the results were reported to be encouraging (Dabaja H & et al., 2020).

In a recent study, Palacio et al. investigated the effectiveness of CO2RE Intima, a vaginal CO2 laser treatment, in treating moderate to severe SUI or mixed urinary incontinence (MUI). They conducted a prospective interventional study at a single center, enrolling 25 women between the ages of 35 and 68 who displayed moderate to severe symptoms of SUI or MUI, as indicated by the Sandvik index and ICIQ-UI scores. The study demonstrated that fractional, micro-ablative CO2RE Intima laser treatment is a minimally invasive procedure that significantly improves moderate and severe SUI and MUI. It is also worth noting that the treatment positively impacts the sexual function of women with these types of incontinence (Palacios & Ramirez, 2020).

Conclusion

The topic of research in the pelvic floor and urinary control field is focused on finding treatment techniques that are less invasive than surgery. These techniques include laser and radiofrequency therapy, periurethral injection therapy, exogenous stem cell therapy, and technology for activating endogenous stem cells. The CO2 laser is an effective, safe, minimally invasive, and well-tolerated approach with no significant complications. However, more research is needed to confirm its efficacy and durability, particularly with a longer follow-up. To consider it as a first-line treatment, robust data is necessary, such as pelvic floor muscle training, or at least as a bridging therapy to surgery.

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Current Treatment of Hypertensive Disorders in Pregnancy

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Hypertensive disorders in pregnancy is one of the major causes of maternal and fetal mortality and morbidity worldwide (Moussa, Arian & Sibai, 2014; Kassebaum et al, 2016). Hypertensive disorders is common in pregnancy affecting %5-10 of all pregnancies (Williams et al, 2018a; Regitz-Zagrosek et al, 2018). Hypertensive disorders lead to increased risk of prematurity, intrauterine growth retardation, stroke, pulmonary edema, thromboembolic events, placental abruption and intrauterine death (Regitz-Zagrosek et al, 2018).

Although there are variabilities between definitions of guidelines, hypertension in pregnancy is traditionally defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, confirmed by a second measurement at least 4 hours apart (or at least 15 minutes if hypertension is severe) (Vidaeff et al, 2019). Hypertension in pregnancy is classified as mild-to-moderate (systolic blood pressure 140-159 mmHg and/or diastolic blood pressure 90-109 mmHg) or severe (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg) hypertension (Williams et al, 2018a). Ambulatory blood pressure monitoring may be more accurate in diagnosing hypertension in pregnancy. However, hypertension in pregnancy is not a homogenous entity.

Hypertensive disorders in pregnancy are classified as; preexisting hypertension, gestational hypertension, preeclampsia, preexisting hypertension plus superimposed gestational hypertension with proteinuria and antenatally unclassifiable hypertension (Regitz-Zagrosek et al, 2018; Williams et al, 2018b).

Preexisting (Chronic) Hypertension:

Hypertension diagnosed before pregnancy or present before 20 weeks of gestation is defined as chronic or preexisting hypertension. Hypertension in pregnancy persisting for at least post-partum 12 weeks, is also defined as chronic hypertension. Chronic hypertension may be masked by vasodilation seen in the first trimester, so diagnose may be challenging. Chronic hypertension is mostly primary hypertension with a family history, however secondary causes may exist. Chronic hypertensive pregnants are at risk of fetal growth retardation, preterm delivery, postpartum hemorrhage and perinatal mortality (Vidaeff et al, 2019). Chronic hypertension is a risk factor of preeclampsia and 20-50% of pregnants with chronic hypertension may experience superimposed preeclampsia (Sibai et al, 1998; Buchbinder et al,2002; Ferrer et al, 2000). Chronic hypertension may be associated with proteinuria (Williams et al, 2018a).

Gestational Hypertension:

Hypertension diagnosed in a pregnant woman after 20 weeks of gestation without proteinuria is called gestational hypertension. It resolves within postpartum 12 weeks. Maternal

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age less than 20 or more than 40 years, preexisting diabetes, nulliparity, African American race, hyperlipidemia, obesity and family history for gestational hypertension are risk factors for developing gestational hypertension (Ros, Cnattingius & Lipworth, 1998). 10-50% of pregnants who have diagnosed as gestational hypertension may develop preeclampsia, especially those diagnosed before 32 weeks of gestation (Saudan et al, 1998; Barton et al, 2001).

Preeclampsia:

Preeclampsia is a systemic disease with new-onset hypertension accompanied by proteinuria or end organ damage after 20 weeks of gestation. International Society for the Study of Hypertension in Pregnancy (ISSHP) defines preeclampsia as hypertension in pregnants after 20 weeks of gestation with concurrent at least one of the following: a) Proteinuria; b) Uteroplacental dysfunction; c) maternal organ dysfunction which includes liver dysfunction (elevated transaminases), hematological findings (trombositopenia <150000/µl, disseminated intravascular coagulation or hemolysis), acute kidney injury (creatinine over 1.1 mg/dl or two fold of basal level), neurological complications (seizures, visual symptoms or headaches) and pulmonary edema (Brown et al, 2018). Preeclampsia is the most severe hypertensive disorder in pregnancy. It is one of the leading causes of maternal, fetal and neonatal adverse outcomes. Preexisting hypertension increase the risk of superimposed preeclampsia by 25% (Seely & Ecker, 2014). The risk of preeclampsia is even more elevated in pregnants who have existing renal disease. Risk factors for preeclampsia is listed in table 1 (Bartsch et al, 2016).

Table 1: Risk factors for preeclampsia.

High risk factors	Moderate risk factors
History of preeclampsia	Maternal age > 40 years
Body mass index ≥30 kg/m²	Nulliparity
Chronic hypertension	Multifetal gestation
Chronic kidney disease	Family history for preeclampsia
Systemic lupus erythematosus or anti-phospholipid syndrome	Prolonged interpregnancy interval (>5years)
Pregestational diabetes mellitus	
Assisted reproductive technology	

Preeclampsia may also present as hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome, which is a severe manifestation of preeclampsia. HELLP accounts for the 5-10% preeclampsias (Fox et al, 2019). In the setting of tonic-clonic generalized, focal or multifocal seizures, another severe manifestation of preeclampsia occurs. This clinical state is defined as eclampsia. While mostly defined in pregnants who have hypertensive disorders, eclampsia may also present as the first symptom. Generally occurs after 28 weeks of gestation, the more being antepartum or intrapartum. Although ISSHP does not classify preeclampsia as

severe or non-severe because of its possibility of rapid deteriorating, severe and non-severe preclampsia is defined by other guidelines (table 2) (Vidaeff et al, 2019).

Prevention:

All pregnants with hypertension should be encouraged for physical activity and healthy diet without significant salt restriction. Aspirin was found to decrease the risk of preeclampsia. In ASPRE (Combined Multimarker Screening and Randomized Patient Treatment with Aspirin for Evidence-Based Preeclampsia Prevention) trial 26941 pregnants were studied (Rolnik et al, 2017). This trial was a double-blind study in which pregnants at risk of developing preeclampsia were divided into two groups. One group was initiated 150 mg aspirin daily while the other group was on placebo starting from 11-14 weeks to 36 weeks of gestation. Aspirin was shown to reduce preterm preeclampsia incidence by 62% in this study. Aspirin has an excellent maternal and fetal safety profile. In a meta-analysis seventy-seven randomised trials with a number of 40249 pregnants were included (Duley et al, 2019). In this meta-analysis; reductions of preeclampsia (sixteen fewer cases per 1000 pregnants treated), preterm birth (sixteen fewer cases per 1000 pregnants treated), baby born small for gestational age (7 fewer per 1000 pregnants treated) and fetal/neonatal deaths (5 fewer per 1000 pregnants treated) were observed in the low-dose aspirin group. Aspirin was shown to decrease severe adverse outcomes by 20 pregnancies in 1000 women treated.

Table 2: Criteria for severe preeclampsia.

Severe blood pressure elevation	Systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥90 mmHg
Nervous system symptoms	Headache unresponsive to analgesics with or without visual symptoms (photopsia, retinal vasospasm)
Hepatic abnormalities	Severe persistent right upper quadrant pain and/or transaminases over two fold of normal range
Renal impairment	Serum creatinine >1.1 mg/dl and/or doubling of baseline serum creatinine
Thrombocytopenia	<100.000 thrombocyte/µl
Pulmonary edema	

Low-dose aspirin (75-162 mg/day) is recommended for prevention of preeclampsia in high risk pregnancies (Brown et al, 2018). High risk for preeclampsia means at least one high-risk factor (chronic hypertension, renal or autoimmune disease, diabetes mellitus, a history of preeclampsia or multifetal pregnancy) or at least two moderate-risk factor (maternal age of ≥35 years, nulliparity, family history for preeclampsia, obesity with BMI >30 kg/m², in-vitro conception, socio-demographic factors like blacks or low income and personal factors like

previous low-birth weight newborn). Aspirin is recommended to be initiated between 12-16 weeks of gestation till delivery.

Treatment of chronic hypertension also reduces the risk of developing preeclampsia (Tita et al, 2022). 1500-2000 mg of calcium intake is recommended in low calcium intake populations for preventing preeclampsia (Hofmeyr et al, 2018). Weight loss before pregnancy and appropriate weight gain during pregnancy may also reduce the risk for preeclampsia (Maggard et al, 2008).

Magnesium sulfate is recommended for preventing progression of severe preeclampsia to eclampsia and seizures (Altman et al, 2002; Duley et al, 2010). 4-6 g of magnesium sulfate should be given as loading dose intravenous over 20-30 minutes. Intravenous infusion of magnesium sulfate should be followed as 1-2 g/hour for a maximum of 24 hours. Respiratory rate, blood pressure, urine output and deep tendon reflexes should be checked every 2 hours to prevent toxicity of magnesium sulfate. Once toxicity is suspected, it is treated by calcium gluconate.

Treatment:

Treatment of non-severe hypertension:

Treatment is recommended in pregnants whose systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. Target blood pressure is recommended as <135/85 mmHg by National Institute for Health and Care Excellence (NICE) and ISSHP guidelines (NICE guidelines, 2019; Brown et al, 2018). Safety issues are restricting drug options in pregnancy. Yet, there are many alternative drugs to use in hypertensive disorders in pregnancy.

Methyldopa: Methyldopa is widely used for years. Methyldopa is central alpha-2 adrenergic agonist. Although its antihypertensive effect is mild, it has the best long-term safety for the fetus. In Control of Hypertension in Pregnancy Study (CHIPS), methyldopa was found to have better outcomes compared to labetalol (Magee et al, 2016). It has a slow onset of action as 3-6 hours. It is initiated as 250 mg 2 or 3 times daily, titrated upto 3000 mg daily in divided doses.

Calcium channel blocking agents: Nifedipine is a dihydropridine calcium channel blocker which is also used in hypertensive disorders in pregnancy for decades. Long-acting form is recommended in long term treatment. The usual starting dose is 30 mg/day titrating upto 120 mg/day.

Amlodipine is also a dihydropridine calcium channel blocker widely used in hypertensive pregnants. A recent meta-analysis has shown that amlodipine is more effective in controling hypertension compared to nifedipine (Yin et al ,2022). Moreover, no significant difference were observed in maternal side effects or pregnancy outcomes in terms of caesarean section, premature labour, abruption of placenta and fetal distress.

Beta blockers: Labetalol is the agent of choice among beta blockers. Labetalol is a non-selective beta blocker with alpha-blocking activity. It has been used widely for years and it may preserve uteroplacental blood flow compared to other beta blockers. While it is rarely seen,

hepatotoxicity may be seen as an adverse effect. Thus, it may be confusing to make a diagnose of HELLP syndrome in pregnants using labetalol. Initial dose for labetalol is 100 mg twice daily upto a total of 2400 mg daily.

Other beta blockers may also be used in pregnants like carvedilol, metaprolol or pindolol. Atenolol and propranolol should be avoided in pregnancy because of lower fetal weight and uterine irritability.

Diuretics: Hydrochlorothiazide and other thiazide-like diuretics may be used in pregnancy when the target blood pressure is not achieved. Although fetal anomalies have not been shown, concerns of hypovolemia limit their use.

Loop diuretics are not recommended in treatment of hypertension, but may be used in pulmonary congestion due to heart failure in pregnancy.

Other antihypertensive agents like angiotensin converting enzyme inhibitors, angiotensin receptor blockers and mineralocorticoid receptor blockers should be avoided in pregnancy for fetal renal abnormalities and other safety issues.

Treatment of severe hypertension in pregnancy:

Severe hypertension is defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg in pregnancy. After confirming blood pressure, antihypertensive treatment should be initiated in 15 minutes to prevent stroke, heart failure or other maternal complications. It is very important that blood pressure should not be agressively treated to <120/80 mmHg, which may reduce uteroplacental blood flow. Mean arterial blood pressure should not be reduced no more than 25% over 2 hours, with the goal being systolic blood pressure 130-150 mmHg and diastolic blood pressure 80-100 mmHg (Cifkova et al, 2020).

Labetalol: Intravenous form of labetalol is the drug of choice where possible, if there is no contraindication like asthma or bradycardia. Onset of action is 1-2 minutes. Initial dose is 10-20 mg intravenous over 5 minutes, with repeat doses of 20 mg in every 20-30 minutes until blood pressure <160/110 mm Hg is achieved. It also may be reasonable to initiate labetalol as intravenous infusion at a rate of 1-2 mg/min. It can be given upto a maximum dose of 300 mg/day. If target blood pressure is not achieved, switching to another agent is recommended.

Hydralazine: Hydralazine is a peripheric vasodilator. Onset of action is 10-20 minutes. Initial dose is 5 mg intravenous or intramuscular over 1-2 minutes, repeating doses of 5-10 mg intravenous every 20 minutes if target blood pressure is not achieved, to a maximum cumulative dose of 20 mg. Alternatively, it can be given as intravenous infusion at a rate of 0.5-10 mg/hour. Hydralazine has less predictable antihypertensive effect compared to labetalol. Maternal hypotension, headaches and abnormal fetal heart rate changes may be seen more common than other agents.

Nifedipine: Immediate release nifedipine is the drug of choice among calcium channel blocking agents. Onset of action is 5-10 minutes. It can be initiated as 10-20 mg orally, followed by a repeat of the dosage in 20 minutes if goal blood pressure is not achieved. Thereafter, 10-

20 mg repeat dose can be given every 2-6 hours, to a maximum daily dose of 180 mg. Headaches and reflex tachycardia may be seen.

Hypertensive emergency in pregnancy is defined as severe hypertension (blood pressure >160/110 mmHg) with progressive acute life threatening end organ damage (acute myocardial infarction, pulmonary edema, aortic dissection, stroke and respiratory failure) (Cifkova et al, 2020). Risk factors are listed in table 3. Treatment options are the same as treatment of severe hypertension. Nicardipine, an intravenous available calcium channel blocker, may also be used in hypertensive emergency. Nicardipine is initiated as 5 mg/hour intravenous infusion, gradually titrated to maximum dose of 15 mg/hour (Cifkova et al, 2020). Pulmonary edema can be treated by adding nitroglycerine infusion starting at a rate of 5 μg/minute, gradually increased every 3-5 minutes to a maximum of 100 μg/minute. It should be used in caution for the risk of cyanide toxicity.

The only definitive treatment for preeclampsia/eclampsia is delivery. In pregnants with gestational hypertension or mild preeclampsia, induction of labour is recommended at 37 weeks of gestation. In women with the features of severe preeclampsia or eclampsia, induction of labour is advised as soon as possible after maternal stability is achieved. Magnesium sulfate treatment added to antihpertensive treatment should be initiated to stabilize patients before delivery. If seizures continue in postpartum patients magnesium sulfate infusion may be extended to a total of 24-48 hours.

Table 3: Risk factors for developing hypertensive emergency in pregnancy.

Chronic renal disease
Preeclampsia
Concomitant use of blood pressure raising agents (cocaine, anabolic steroids)
Use of uterocontractive agents (ergonovine maleat)
Non-compliance to antihypertensive agents
Cardiac disease
Low socioeconomic status
Non-Hispanic blacks

Hypertension usually disappears within a few weeks postpartum. Close monitoring is recommended during this period, particularly those who are on antihypertensive treatment. After 12 weeks after delivery, gestational hypertension should be resolved.

Antihypertensive drug choices are similar during breastfeeding. Methyldopa and hydralazine appear to be safe for newborns during breastfeeding. Although beta blockers like labetalol and metaprolol or calcium channel blockers like nifedipine enter into breast milk, they appear to be safe for infants. Angiotensin converting enzyme inhibitors are transferred into

breast milk at very low levels. However, the effects of this group are not clear in infants. Diuretics are not recommended for concerns about decreased milk production.

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Torque Teno Virus, Hepatitis Infections and Hepatocellular Carcinoma

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Giriş

The Torque teno virus (TTV) was isolated in Japan at the end of 1997 from a serum of an acute post transfusion hepatitis patient with unknown etiology (Nishizawa & et al., 1997). In the beginning, the TT virus was identified in Parvoviridae family. The International Committee on Taxonomy of Viruses at first did not officially classify the virus; however the latest 2015 taxonomy reveals it in Anelloviridae family (Lefkowitz & et al., 2018). Healthy individuals from various countries are reported to have a variety of (1–95%) TTV DNA positivity rate (Allain & et al., 2009; Najafimemar & et al., 2018; Sarairah, Bdour & Gharaibeh, 2020). According to reports, TTV affects 90% of the general population and can be found in peripheral blood, stools, saliva, cerebral fluid, and pharyngeal mucus (Emmel & et al., 2020; Pou & et al., 2018). Previous research has shown a link between TTV viral load and immune system deficits brought on by persistent infections and cancer (Spandole & et al.,2015; Okamoto, 2009). Also, the relation of TTV with liver diseases and respiratory tract malfunctions, its co-infection with other pathogens, its relation with immune suppressed diseases/patients, its relation with cancer, autoimmune and hematologic diseases were revealed (Giacconi & et al., 2018; Reshetnyak & et al., 2020; Mrzljak & Vilibic, 2020). Additionally, many studies have been carried out stating the TTV's co-infection with hepatitis viruses (mainly Hepatitis B Virus and Hepatitis C Virus). Though, the existence of hepatocellular carcinoma (also called malignant hepatoma) and HCV related chronic liver disease are reported to be related with high TTV load, the TTV cannot be explained whether it is a co-factor or it is the cause of the disease (Rosa & et al., 2017). In this paper, we provide a book chapter of currently currently available studies on the relationship between TTV and hepatitis infections and TTV and liver related cancers especially hepatocellular carcinoma.

Bu kitap bölümü Prof. Dr. M. Esra KOÇOĞLU danışmanlığında 20.01.2012 tarihinde tamamladığımız "Sağlıklı Kan Donörlerinde Transfüzyonla Geçen Virüs (TT Virüs) Sıklığının Araştırılması" başlıklı yüksek lisans tezi esas alınarak hazırlanmıştır (Yüksek Lisans Tezi, Bolu Abant İzzet Baysal Üniversitesi, Bolu, Türkiye, 2012). / This book chapter is extracted from my master thesis entitled "Investigation of the Frequency of Transfused Virus (TT Virus) in Healthy Blood Donors", supervised by Prof. Dr. M. Esra KOÇOĞLU (Master's Thesis, Bolu Abant İzzet Baysal University, Bolu, Turkey, 2012).

Torque Teno Virus (TTV)

Small single stranded, icosahedral, and unenveloped DNA viruses known as torque teno viruses (TTVs) are members of the Anelloviridae family. TTV was isolated from a patient's serum with acute posttransfusion hepatitis of unknown etiology in Japan at the end of 1997. It was previously thought to be responsible for hepatitis developing after transfusion, and some people used this abbreviation to mean transfusion-transmitted virus. Nishizawa and his

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colleagues stated that they used the initials of the patient's name when naming this new virus they discovered (Nishizawa & et al., 1997). Later, TTV was also used as the Torque teno virus (Latin "torques," necklace; "tenuis," thin) (Öz, Adıgül & Altındiş, 2018; Rabelo & et al., 2023). Along with two other viruses—the torque teno micro virus and torque teno midi virüs-which were given their diminutive genome sizes, TTV is a member of the Anellovirus family (Lefkowitz & et al., 2018).

General Properties

The original 500 base length DNA clone in which the TTV was first identified was named DNA N22. By using the primers obtained from this clone, a TTV isolate (TA278) was found in the plasma of a blood donor with high level ALT (Okamoto, 2009; Oliveira, 2015). In 1998, Okamoto defined the virus genome as a DNA with 3739 nucleotides and a single spiral. At first, two open reading frames (ORF1 and ORF2) and then three ORFs were revealed to exist. Another group from Western Africa reported a TTV isolate genome, which they named a nucleotide series, to have circular, negatively polarized, 3852 nucleotides (Okamoto & et al., 1998). TTV was first thought to be related to the Parvoviridae family due to sequence analysis. Then it was suggested to examine it within the Anelloviridae family due to the similarity of its genome consisting of circular DNA and genomic features (Figure 1, Table 1) (Itoh & et al., 2000). Based on phylogenetic research, five primary genetic groups (Groups 1-5) involving at least 39 genotypes have been discovered thus far (Oliveira, 2015). The TTV, torque teno mini virus (TTMV), and torque teno midi virus (TTMDV), which have high genomic variability, were found after the TTV (Figure 2) (Hino & Miyata, 2007).

Table 1. Current Anelloviridae family (Lefkowitz & et al., 2018)

Species	**Genus**	**Host**
Torque teno virus	*Alphatorquevirus*	Human, chimpanzee
Torque teno mini virus	*Betatorquevirus*	Human, non-human primate
Torque teno tupia virus	*Deltatorquevirus*	Tupaias
Torque teno tamarin virus	*Epsilontorquevirus*	Tamarin
Torque teno felis virus	*Etatorquevirus*	Cat
Torque teno midi virus	*Gammatorquevirus*	Human, chimpanzee
Chicken anemia virus	*Gyrovirus*	Chicken
Torque teno sus virus 1	*Iotatorquevirus*	Swine
Torque teno sus virus k2	*Kappatorquevirus*	Swine
Torque teno zalophus virus 1	*Lambdatorquevirus*	Sea lion
Torque teno canis virus	*Thetatorquevirus*	Dog
Torque teno douroucouli virus	*Zetatorquevirus*	Douroucouli

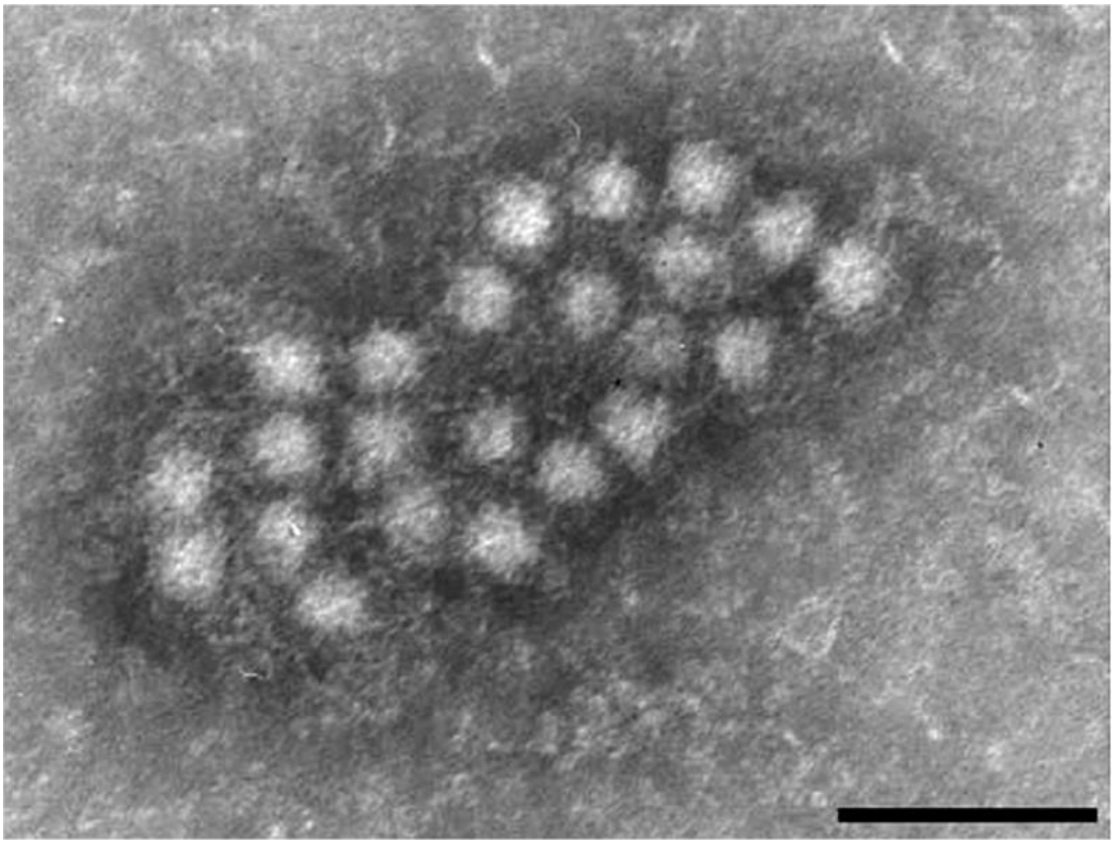

Figure 1. Appearance of TTV in the electron microscope. The bar represents 100 nm (Itoh & et al. 2000).

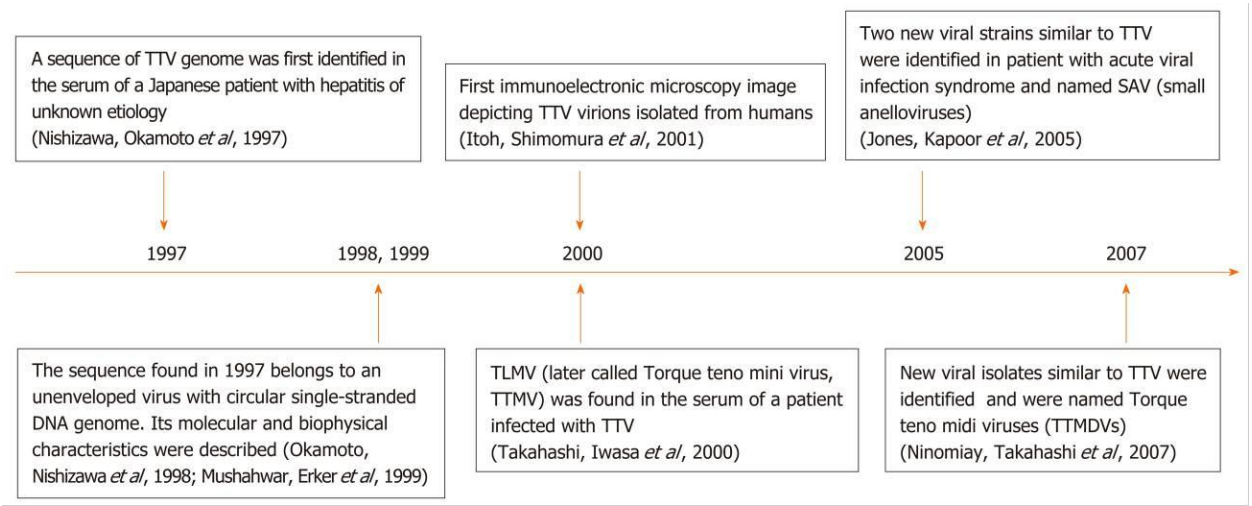

Figure 2. The first steps in researching the Anelloviridae family, TTV: Torque Teno Virus (Spandole & et al. 2015).

The genome contains a coding region of approximately 2.6 kb and the remaining 1.2 kb a non-coding region (UTR). Around 110 nucleotides of the non-coding region are rich in Guanine (G) and Cytosine (C), and this region has been shown to complement circular DNA (Figure 3) (King & et al., 2012).

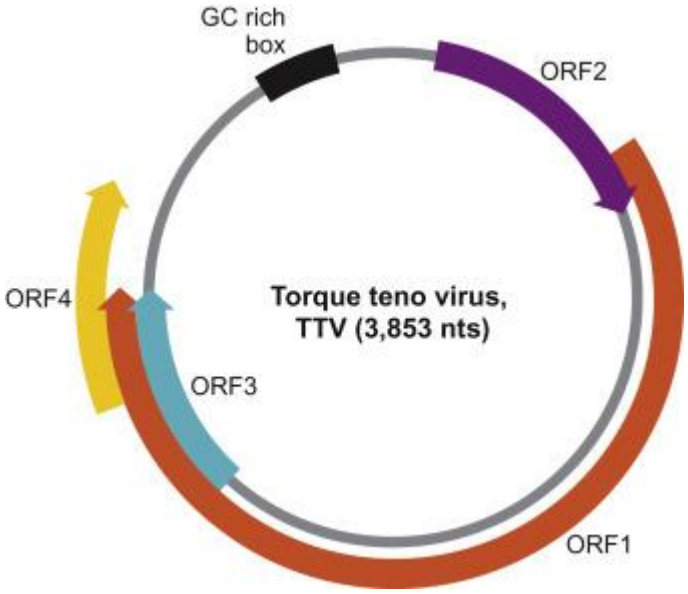

Figure 3. Genome organization of TTV (King & et al., 2012).

Due to the virus's great prevalence, which renders it nearly universal in the human population and able to elude host immune response clearance, long-term chronic infections can develop. The short 22 nt noncoding RNAs known as microRNAs (miRNAs), which have been identified as key regulators of gene expression in many eukaryotes and even viruses, can be highlighted in this context. Immune evasion, extending the lifespan of host cells, and controlling chronic infection are emerging themes of viral miRNA action (Boss & Renne, 2010; Kincaid & et al., 2013).

TT Virus-Host Relationship and Clinical Features

TTV is a small, circular, single-stranded DNA virus that is part of the Anelloviridae family. While TTV is generally considered nonpathogenic and asymptomatic in most cases, it is involved in complex interactions with its host, the human body. Scientists from all over the world have been working on the problem of studying TTV for the past two decades. TTV is a virus that is frequently found in patients with various viral hepatitides, in cases of hepatitis without an obvious viral agent, as well as among a healthy population. These pathogen-host interactions are of significant interest to researchers. Here's an overview of some key aspects of TTV's interactions with its host:

High Prevalence and Persistence: TTV is highly prevalent in the human population worldwide. It can be found in various bodily fluids, including blood, saliva, and feces. Many people carry the virus asymptomatically, and it often persists in the host for extended periods, potentially throughout a person's life and most carriers of the virüs do noy experience any clinical symptoms or signs of illness. The mechanisms through which TTV establishes and maintains persistence are not fully understood (Oliveira, 2015; Reshetnyak & et al., 2020).

Host Immune Response: TTV activates both the innate and adaptive immune responses of the host. The virus's presence in the bloodstream can trigger the release of immune signaling molecules, including interferons and proinflammatory cytokines. TTV-specific T cell responses have been observed in individuals with the virus, indicating the host's ability to recognize and respond to TTV (Maggi & Bendinelli, 2009).

Immunomodulation: TTV may employ immunomodulatory strategies to evade the host immune response. For example, it can interfere with the host's interferon response, which is critical for antiviral immunity. By dampening the interferon response, TTV may reduce the host's ability to control the infection and establish persistence (Maggi & Bendinelli, 2009; Studenic & et al., 2022).

Genetic Diversity: TTV is known for its genetic diversity, with multiple genotypes and variants. This genetic variability is thought to play a role in the virus's ability to evade immune clearance. Different TTV strains may have distinct characteristics, and the host immune response generated against one strain may not be effective against other strains (Hino & Miyata, 2007; Cortey & et al., 2011).

Latent or Persistent Infection: TTV is often described as a latent or persistent virus, which means it can persist in the host without causing acute symptoms. The mechanisms that allow TTV to maintain a latent or persistent state in the host are not fully understood. It's likely a combination of factors, including genetic diversity and immunomodulation (Redondo & et al., 2022; Oliveira, 2015).

Co-infections: TTV is frequently found in individuals with other viral infections, such as hepatitis B and C. Co-infections with TTV and other viruses can complicate the host's immune response, as these viruses may interact and influence one another within the host (Prasetyo, Dharmawan & Raharjo, 2016; Ghosh & et al., 2020).

Role in Disease: While TTV itself is not considered a direct cause of disease, some studies have explored its potential associations with various health conditions, including liver diseases and cancer. However, the exact nature of these associations is still a subject of ongoing research. TTV's pathogen-host interactions are multifaceted. The virus interacts with the host's immune system, establishes persistence, and employs various mechanisms to evade immune clearance. Research into TTV's role in human health and disease is ongoing, and scientists continue to explore the intricacies of these interactions to gain a deeper understanding of the virus's impact on the host (Maggi & Bendinelli, 2009; Hino & Miyata, 2007; Reshetnyak & et al., 2020; Webb, Rakibuzzaman & Ramamoorthy, 2020).

TTV infection is cleared from the host over time. It is not known by what mechanism it is removed. Host- or virus-related characteristics, or a combination of the two, may be influential. No recurrence of viremia was observed in TTV DNA-negative patients in studies. Virus carriers in experimentally infected chimpanzees disappeared after months. Okamoto et al. Like other single-stranded DNA viruses, they thought TTV DNA could act as an episome and integrate into the host's DNA. Still, studies showed that TTV was not incorporated in patients with hepatocellular and hematopoietic malignancies (Sarairah, Bdour & Gharaibeh, 2020; Okamoto, Nishizawa & Takahashi, 2003).

Patogenesis and Patology

The tissues and organs in which TTV carries out its primary multiplication after entering the body and the target tissues of the virus have yet to be precisely defined. There is no suitable experimental animal or cell culture model to examine the pathogenesis of viral infection. DNA of TT virus has been shown in serum and plasma samples, peripheral blood mononuclear cells, liver, spleen, kidney, lung tissues, central nervous system, gastric and intestinal mucosa. However, it needs to be clarified whether replication occurs in all of these organs. In addition,

nucleic acids belonging to the virus have been detected in saliva, stool, hair, skin scrapings, nasopharyngeal secretions, tears, semen, breast milk, and cord blood (Lolomadze & Rebrikov, 2020; Webb, Rakibuzzaman & Ramamoorthy, 2020). TTV DNA is found at high rates in different non-A-G hepatitis patient populations. Although it has been suggested that TTV may be a factor due to the detection of TTV DNA in the serum in cases where pre-transfusion TTV is negative and acute post-transfusion hepatitis develops, it has not been proven. Although studies show the relationship of TTV with acute and persistent infections, its clinical significance has not been explained (Webb, Rakibuzzaman & Ramamoorthy, 2020; Spandole & et al.,2015). In recent studies, most TTV-positive individuals have no biochemical or histological findings indicating significant liver damage. There is no significant difference in histological activity or ALT levels in the liver between TTV DNA-positive and negative cases (Reshetnyak & et al., 2020; Piaggio & et al., 2009).

In prospectively monitored patients, the rate of new TTV infections was similar in those who developed acute hepatitis and those who did not. These data support the view that TTV infection alone does not cause significant liver damage but may be accompanied by another hepatitis agent. Since there is no difference in the severity of the disease between positive and negative TTV in HBV or HCV infections, it is believed that it does not affect increasing liver damage. The hepatotrophic feature of the virus is accepted due to the detection of TTV DNA (10-100 times more than in serum samples) and double-stranded DNA, which may be an indicator of replication in the liver tissues of infected people (Reshetnyak & et al., 2020; Alavi & et al., 2011; Jalali, Mahdavi & Zaeromali, 2017). However, the absence of morphological changes in hepatocytes indicates that TTV does not have an essential role in liver pathogenesis. Additionally, its role in hepatitis or exacerbating liver damage has not been proven (Hazanudin & et al., 2019; Reshetnyak & et al., 2020; Najafimemar & et al., 2018). After the injection of TTV-positive serum into two chimpanzees, acute TTV infection was detected; TTV DNA nucleic acid sequences in the inoculate were the same as those in chimpanzees. However, no histological or biochemical evidence of hepatitis was found in either chimpanzee. The relationship between TTV genotypes and liver disease is also being investigated, but no significant relationship has been found yet (Hino & Miyata, 2017; ninomiya & et al., 2007).

Hepatitis B Virus (HBV)

Hepatitis B Virus (HBV) is a viral infection that primarily affects the liver. It is a member of the Hepadnaviridae family. HBV infection can lead to both acute and chronic liver disease, and it is a significant global health concern. Here are key characteristics and information about the hepatitis B virüs (Dusheiko, Agarwall & Maini, 2023; Tang & et al., 2018; Tu & Douglas, 2020):

Overview-Viral structure: Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic diseases. It is transmitted through contact with the blood or other body fluids of an infected person. The virus can lead to severe liver conditions such as cirrhosis and liver cancer. HBV is a partially double-stranded DNA virüs. It has an outer envelope, which contains surface proteins, and an inner core that houses the viral DNA and associated proteins.

Prevalence: Worldwide, an estimated 257 million people were living with chronic HBV infection as of 2019. The prevalence varies significantly by region, with higher rates in parts of Africa and Asia.

Acute vs. Chronic Infection: HBV infection can be either acute or chronic. Acute infections are usually short-lived and self-limiting. Chronic infections, on the other hand, persist for six months or longer and can lead to long-term liver complications, such as cirrhosis and liver cancer.

Vaccination: There is an effective vaccine for Hepatitis B that is recommended for all infants and is commonly administered in three doses during childhood.

Symptoms: Acute HBV infection may result in symptoms such as jaundice, fatigue, abdominal pain, and loss of appetite. However, many individuals with acute infection are asymptomatic. Chronic HBV infection may not cause noticeable symptoms for years but can lead to severe liver damage over time.

Diagnosis: Blood tests can detect the presence of HBV, its viral load, and the status of infection (acute or chronic). Screening is essential to identify and manage infected individuals.

Treatment: Antiviral medications are available to manage chronic Hepatitis B and reduce the risk of complications. The goal of treatment is to suppress the virus and prevent liver damage.

Transmission: HBV is spread through contact with the blood or other body fluids of an infected person, such as through sexual contact, sharing needles, or from mother to child during childbirth.

Prevention: Preventative measures include vaccination, practicing safe sex, not sharing needles or drug paraphernalia, and ensuring safe blood transfusions and medical procedures. Infants born to mothers with HBV should receive prophylactic treatment to prevent transmission.

Complications: Chronic HBV infection can lead to serious complications, including cirrhosis of the liver and an increased risk of liver cancer. Early diagnosis and appropriate medical care are crucial in managing these risks.

Hepatitis C Virus (HCV)

Hepatitis C virus (HCV) is a viral infection that primarily affects the liver. It is a member of the Flaviviridae family. HCV infection can lead to both acute and chronic liver disease, and it is a significant global health concern. Here are key characteristics and information about the hepatitis C virüs (Busschots & et al., 2022; Shahid & et al., 2021; Elgretli & et al. 2023):

Overwiew-Viral Structure: Hepatitis C is another viral infection that primarily affects the liver. It can result in both acute and chronic liver disease and is often asymptomatic in its early stages. HCV is a single-stranded RNA virus. It has a high degree of genetic diversity, with multiple genotypes and subtypes. This diversity can affect the course and response to treatment of HCV infection.

Prevalence: HCV is a global health concern, approximately 71 million people had chronic HCV infection worldwide as of 2015. The prevalence of HCV varies by region, with high rates in some countries.

Transmission: HCV is primarily transmitted through contact with the blood of an infected person. Common modes of transmission include sharing needles for drug use, exposure

to contaminated blood or blood products, and, less commonly, through sexual contact. Vertical transmission from mother to child during childbirth is also possible but less common than with hepatitis B.

Acute vs. Chronic Infection: HCV infection can be either acute or chronic. Acute infections are often asymptomatic or result in mild, flu-like symptoms and are usually self-limiting. However, a significant proportion of individuals develop chronic HCV infection, which can lead to long-term liver complications.

Symptoms: Acute HCV infection may result in symptoms such as fatigue, jaundice, and abdominal pain. However, many individuals with acute infection are asymptomatic. Chronic HCV infection may not cause noticeable symptoms for years, but it can lead to severe liver damage over time.

Diagnosis: Blood tests can detect the presence of HCV and determine the viral load. Screening and diagnostic tests are essential to identify and manage infected individuals.

Treatment: In recent years, significant advancements have been made in the treatment of HCV. Direct-acting antiviral drugs (DAAs) have revolutionized HCV therapy, offering a high cure rate with relatively few side effects. Treatment duration and specific medications may vary depending on the genotype of the virus.

Prevention: Preventative measures include harm reduction strategies for people who inject drugs, practicing safe sex, not sharing needles or drug paraphernalia, and ensuring safe blood transfusions and medical procedures.

Complications: Chronic HCV infection can lead to serious complications, including cirrhosis of the liver, liver cancer, and liver failure. Early diagnosis and appropriate medical care are crucial in managing these risks.

Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and is a significant global health concern. HCC primarily develops in the hepatocytes, the main functional cells of the liver, and it is associated with underlying liver diseases (Ghouri, Mian & Rowe, 2017; Balogh & et al., 2016). The most common risk factor for HCC is chronic hepatitis infection, especially HBV and HCV. These viral infections can lead to liver inflammation, fibrosis, and cirrhosis, which increase the risk of HCC (Janevska, Chaloska-Ivanova & Janevski, 2017). Cirrhosis, a late-stage scarring of the liver often caused by alcohol abuse, chronic viral hepatitis, or other conditions, is a significant risk factor for HCC. Most HCC cases occur in individuals with cirrhosis (N'Kontchou & et al, 2006). Heavy and prolonged alcohol consumption can damage the liver and increase the risk of HCC, especially when combined with other risk factors. Nonalcoholic Fatty Liver Disease (NAFLD) is a condition associated with obesity and metabolic syndrome, can progress to nonalcoholic steatohepatitis (NASH) and cirrhosis, increasing the risk of HCC (Zoller & Tilg, 2016). In some parts of the world, exposure to aflatoxins, toxic substances produced by certain molds, can increase the risk of HCC. Hereditary conditions like hemochromatosis, which leads to excess iron buildup in the liver, can elevate the risk of HCC. Obesity is associated with an increased risk of HCC, often due to its role in causing fatty liver disease and insulin resistance (Janevska, Chaloska-Ivanova & Janevski, 2017; Schütte, Bornschein & Malfertheiner, 2009).

HCC may not exhibit noticeable symptoms in its early stages. As it advances, symptoms can include abdominal pain, unexplained weight loss, jaundice (yellowing of the skin and eyes), and a feeling of fullness or discomfort in the upper abdomen. Diagnosis typically involves imaging studies such as ultrasound, CT scans, or MRI, along with blood tests to assess liver function and identify tumor markers like alpha-fetoprotein (AFP). Confirmatory diagnosis is achieved through a liver biopsy, where a small sample of liver tissue is examined under a microscope. The choice of treatment for HCC depends on the stage of the cancer, the patient's overall health, and the underlying liver condition. Treatment options may include surgical resection, liver transplantation, ablation therapies (radiofrequency or microwave ablation), transarterial chemoembolization (TACE), targeted therapy, and immunotherapy. Prognosis for HCC varies significantly, with early-stage tumors having a better prognosis. Late-stage HCC has a higher risk of recurrence and metastasis, which makes it more challenging to treat successfully. Liver transplantation can be a curative option for select patients with advanced HCC. Prevention strategies include getting vaccinated against hepatitis B, avoiding high-risk behaviors that lead to hepatitis infections, reducing alcohol consumption, maintaining a healthy weight, and managing underlying liver diseases. Regular surveillance for individuals at high risk of HCC is crucial for early detection and intervention (Attwa & El-Etreby, 2015; Sun & Sarna, 2008; Yang & et al., 2019).

HCC is a complex and aggressive disease, often associated with underlying liver conditions. Timely diagnosis and appropriate management are critical in improving outcomes for individuals affected by HCC. Advancements in early detection and treatment approaches are key to addressing the challenges posed by this form of liver cancer.

The Association between TTV and HCC

The association between TTV and HCC is a subject of ongoing research and debate in the scientific community. TTV is a relatively newly discovered virus that is often asymptomatic and has been found in the blood of a significant portion of the human population. While TTV is generally considered nonpathogenic and harmless, some studies have suggested potential links between TTV and liver diseases, including HCC. Some research studies have reported a higher prevalence of TTV in individuals with HCC compared to those without liver cancer. However, this association does not establish a causative relationship, and other factors may be at play (Yoshida & et al., 2000; Tokita & et al., 2002).

While there appears to be a correlation between TTV and HCC, it's essential to distinguish between correlation and causation. The presence of TTV in HCC patients may not necessarily mean that TTV directly causes HCC. Other factors, such as chronic hepatitis B or C infections, cirrhosis, and environmental factors, also play a significant role in HCC development. HCC typically arises in the context of chronic liver diseases and cirrhosis. TTV is often detected in individuals with underlying liver conditions, which can complicate efforts to determine its specific role in HCC. TTV infections may be more common and potentially cause complications in individuals with compromised immune systems, such as organ transplant recipients or those with HIV/AIDS. This suggests that TTV's impact may vary in different populations. The exact mechanisms by which TTV, if at all, may contribute to liver disease progression or HCC are not fully understood. Some hypotheses include immune system modulation, potential indirect effects on liver health, or interactions with other viruses (Tangkijvanich & et al., 1999; Tokita & et al., 2002).

In summary, while some studies have reported a correlation between TTV and HCC, the exact nature of this association remains unclear, and it is a topic of ongoing research. TTV is not considered a primary cause of HCC, and the development of HCC is a multifactorial process often involving other well-established risk factors, such as chronic hepatitis B or C infections. Further research is needed to better understand the significance of TTV in the context of HCC and liver diseases. For individuals with concerns about TTV and its potential impact on liver health, consulting with healthcare professionals is advisable to receive the most up-to-date information and guidance based on their specific medical situation.

Relationship of TTV with Liver Diseases

While TTV itself is generally considered harmless, it has been found to be more prevalent in individuals with certain liver diseases, such as hepatitis and cirrhosis. Some studies have suggested that TTV may play a role in liver disease progression. TTV infections are often found in individuals who are co-infected with HBV or HCV. The presence of TTV has been associated with more severe liver disease in some individuals with chronic hepatitis B or C (Reshetnyak & et al., 2020). TTV infections may be more common and potentially cause complications in individuals with compromised immune systems, such as organ transplant recipients or those with HIV/AIDS (Garcia-Alvarez & et al., 2013). The exact role of TTV in liver disease progression remains a subject of ongoing research. It is not clear whether TTV directly contributes to liver damage or if its increased presence is a consequence of liver disease. TTV has been explored as a potential diagnostic marker for liver diseases, particularly in individuals with chronic hepatitis. Elevated TTV levels have been associated with more advanced liver fibrosis (Asim & et al., 2010; Reshetnyak & et al., 2020).

It has been emphasized in the literature that the TTV viremia level of patients with hepatitis may be high, and the high prevalence of the virus in the hepatitis patient group has been held responsible for this (Pistello & et al., 2001). It cannot be fully explained whether TTV replication is the cause or consequence of liver damage. There have been many studies on the co-infection of TTV with other hepatitis viruses (mainly HBV and HCV). Although HCV-related chronic liver disease and the presence of HCC are associated with high TTV burden, it is not clear whether TTV is a cofactor of the disease or the cause of the disease. Different studies have emphasized that co-infection with TTV does not contribute to severe hepatitis or other hepatic damage due to HBV or HCV (Burra & et al., 2008; Nobili & et al., 2005). In summary, it is stated that TTV may be associated with liver diseases such as cryptogenic chronic liver disease, transfusion-related hepatitis, liver cirrhosis, fulminant hepatitis, HCC, and alcohol-related liver diseases. It has been emphasized that there may be a relationship between different hepatic disorders the frequency of TTV, and the viral load of TTV.

Co-infection of TTV with The Other Diseases

Relationship with respiratory disorders

It has been shown that TTV infection plays an active or opportunistic role in acute respiratory diseases in children (Maggi & et al., 2003a). In addition, it has been demonstrated that a high TTV burden contributes to the pathogenesis of asthma in children (Pifferi & et al., 2005). Interestingly, it has been shown that TTV replication also occurs in lung tissues, and high TTV load is associated with severe bronchiectasis (Bando & et al., 2001; Pifferi & et al. 2006).

Co-infection with other pathogens

Co-infection of TTV with Human Papilloma Virus (HPV) has been demonstrated (Siahpoush & et al., 2022). It has been suggested that high TTV load is also found in the gastric tissues of patients with gastritis and that *Helicobacter pylori* plays a role in TTV infection (Maggi & et al., 2003b).

Relationship with immunosuppressed diseases/patients:

Some genotypes of TTV are more common in HIV-infected patients than in healthy individuals (Schmidt & et al., 2021; Sherman, Rouster & Feinberg, 2001).

Relationship with cancer:

It has been shown that TTV DNA is found in different types of lymphoma (Hodgkin's disease) and is localized to non-neoplastic cells (Garbuglia & et al., 2003). Zur Hausen et al. showed in their study that TTV infection affects the development of childhood leukemia and lymphoma (Zur Hausen & de Villiers, 2005). Co-infection of HPV and TTV genotype one is associated with laryngeal cancer, but it is unknown whether TTV affects cancer progression. It has been stated that TTV plays a role in the formation of colorectal cancer, but the same TTV isolate is also found in non-cancerous tissues. TTV DNA has also been found in patients with classical Kaposi's sarcoma serum. It has been suggested that TTV affects the pathogenesis of HHV8 in immunosuppressed conditions (Girard & et al., 2007; Kronenberg & et al., 2022).

Relationship with autoimmune diseases:

It has been stated that TTV also plays a role in autoimmune diseases such as Systemic lupus erythematosus (SLE), Multiple sclerosis (MS), Rheumatoid arthritis (RA), Idiopathic inflammatory myopathy (IIM). Sospedra et al. Their study showed that T cells obtained from MS patients reacted against arginine-rich 10 TTV and peptides similar to the ORF1 N terminus of TTMV. They suggested that the recurrence of TTV infections leading to T cell stimulation and some genetic and/or microbial predisposing factors may play a role in developing this type of autoimmune disease (Sospedra & et al., 2005). Whether TTV has a role in autoantibody production or causes immunological dysfunction has not been elucidated. It has been stated that it may be possible that those with autoimmune diseases are more prone to TTV infections. In addition, researchers suggest that there may be a relationship between TTV replication and arthritis. However, considering the other disorders mentioned, their causes are still unknown (Gergely, Perl & Poór, 2006; Mancuso & et al., 2013; Maggi & et al., 2007; Costa & et al., 2012).

Relationship with hematological diseases:

Although different studies have shown that TTV is associated with aplastic anemia, there are also studies stating that it has no relationship with post-hepatitis aplastic anemia (Maeda & et al., 2000; Kanda & Hirai, 2001).

It's important to note that while TTV's role in liver diseases is of scientific interest, it is not considered a primary cause of liver diseases. Rather, TTV's presence in individuals with liver diseases may reflect the underlying liver damage and impaired immune response in these patients. Research on the relationship between TTV and liver diseases is ongoing, and the understanding of its significance continues to evolve. If you have concerns about TTV and its

connection to liver disease, it is advisable to consult with a healthcare professional who can provide the most up-to-date information and guidance based on your specific health situation.

TTV and Immunology

TTV is a relatively newly discovered virus, and research on its immunology is an ongoing area of study. TTV belongs to the Anelloviridae family and is characterized by its high prevalence in the human population. Here are some key points about TTV and its immunology:

Innate Immune Response: TTV, like other viruses, can activate the host's innate immune response. This involves the recognition of viral components, such as TTV DNA, by pattern recognition receptors (PRRs) like Toll-like receptors (TLRs). Activation of these receptors leads to the production of interferons and proinflammatory cytokines as part of the antiviral response.

Adaptive Immunity: The role of adaptive immunity, particularly T cell responses, in TTV infection is an area of ongoing research. TTV-specific T cell responses have been detected in individuals with the virus, indicating that the host immune system recognizes TTV and attempts to control it.

Immune Evasion: TTV, like many persistent viruses, may employ mechanisms to evade the host immune response. For instance, it may interfere with the host's interferon response, which is crucial for antiviral immunity. This interference can dampen the host's ability to control the infection.

Immunomodulation: TTV's interactions with the immune system are complex and not fully understood. It may employ immunomodulatory strategies to limit the host's immune response, though the exact mechanisms are still being elucidated.

Chronic Infection: TTV often establishes chronic or persistent infections in individuals. The host immune response's role in maintaining these long-term infections and the factors contributing to immune control or evasion are subjects of active investigation.

Genetic Diversity: TTV has a high degree of genetic diversity, with multiple genotypes and variants. This diversity can impact the host's immune response, as different strains of TTV may have distinct characteristics and may interact differently with the host's immune system.

Clinical Implications: The clinical implications of TTV and its interactions with the immune system remain areas of ongoing research. While TTV is generally considered nonpathogenic and often asymptomatic, its potential associations with certain health conditions are being explored, including liver diseases and cancer.

In summary, research into the immunology of Torque teno virus is a dynamic and evolving field. While there is a growing understanding of how TTV interacts with the human immune system, many aspects of these interactions, including their clinical significance, are still being investigated. For the most current and detailed information on TTV immunology, it is advisable to refer to recent scientific literature and research findings in the field of virology and immunology (Maggi & Bendinelli, 2009; Maggi & et al., 2011; Webb, Rakibuzzaman & Ramamoorthy, 2020; Kakkola, 2008).

Immune Response

Since it is known that the virus can be cleared spontaneously in acute and chronic infections, antibody tests are needed to give the TTV exposure rate. However, reliable TTV antibody tests have yet to be developed. Tsuda et al. reported that anti-TTV antibodies identified by the immunoprecipitation method could be found in TTV-positive and negative cases (Tsuda & et al., 1999). It has been shown that in some cases, TTV DNA disappears with the development of antibodies. However, more information about these antibodies' meaning and properties needs to be provided. In addition, the high genetic and amino acid variability in TTV may constitute mechanisms to evade the immune response. Thus, it may not protect against recurrent infections. The role of immune complexes and cellular immune response in the pathogenesis of TTV infection is also unknown (Maggi & Bendinelli, 2009; Oliveira, 2015).

TTV and its immune response was limited due to the relatively limited research conducted on this virus. TTV is generally considered nonpathogenic and often asymptomatic, and its interactions with the human immune system were not as extensively studied as other viruses like hepatitis B or C.

The Recommendations for New Studies on TTV

Research on TTV is ongoing, and there are several areas where new studies can contribute to a better understanding of this virus and its implications for human health. Some recommendations for future studies on TTV include:

- Investigate the prevalence and distribution of TTV genotypes and variants in different populations and geographic regions. Understanding the global epidemiology of TTV can provide insights into its transmission dynamics and potential public health implications.
- Study the immune responses to TTV, both innate and adaptive, and explore how TTV modulates the host immune system. This can help clarify the immunological role of TTV and its impact on the host.
- Investigate potential associations between TTV and various health conditions, including liver diseases, autoimmune diseases, and other chronic illnesses. Determine whether TTV plays a direct or indirect role in the pathogenesis of these conditions.
- Further explore the genetic diversity of TTV, including how different TTV genotypes and variants affect the host and whether they are associated with distinct clinical outcomes.
- Investigate the molecular biology of TTV, including its replication, transcription, and potential protein products. Elucidating the life cycle of TTV can provide insights into its persistence and potential pathogenic mechanisms.
- Develop animal models for TTV to facilitate in vivo studies of its pathogenicity, host interactions, and potential clinical implications.
- Investigate the mechanisms through which TTV may contribute to disease, such as its impact on liver function or the development of liver cancer.
- Develop more sensitive and specific diagnostic methods for TTV, which can aid in both research and clinical applications.
- Explore potential preventive strategies for TTV infections, particularly in individuals with compromised immune systems, where TTV may lead to complications.
- Conduct long-term, prospective studies to track TTV infections and their outcomes in diverse populations, focusing on individuals with chronic diseases or immunosuppression.

- Investigate potential antiviral or immunomodulatory therapies for TTV, especially in cases where TTV is linked to specific diseases.
- Assess the public health impact of TTV, including its potential association with liver disease and other health conditions. Determine whether monitoring TTV infections can aid in disease prevention or management.

Continued research into TTV is essential to elucidate its role in human health and disease. As our understanding of TTV advances, it may lead to better diagnostic tools, preventive measures, and potential therapeutic interventions for individuals who may be at risk or affected by TTV-related conditions.

Conclusion

In conclusion, TTV is a widely prevalent and relatively newly discovered virus with complex interactions with the human immune system. While TTV is generally considered nonpathogenic and often asymptomatic, its precise role in human health and disease remains a subject of ongoing research. TTV's genetic diversity, ability to establish persistent infections, and its associations with certain health conditions, including liver diseases and HCC, have sparked scientific interest. Hepatitis infections, particularly those caused by HBV and HCV, are major global health concerns. These viruses can lead to both acute and chronic liver diseases, ranging from mild to severe, and may culminate in life-threatening complications such as cirrhosis and HCC. The development of vaccines, antiviral treatments, and prevention strategies has been pivotal in reducing the burden of these infections. However, challenges remain in diagnosis, treatment accessibility, and ongoing efforts to eradicate these viral infections. HCC, the most common form of primary liver cancer, is often associated with chronic hepatitis infections, especially HCV. It is a complex and aggressive disease with a high mortality rate. Early detection and management of underlying liver conditions, along with ongoing research into new treatments and prevention strategies, are crucial in addressing the HCC burden. In the case of TTV, ongoing research is shedding light on its immunological interactions and potential associations with liver diseases and other health conditions. The complex relationship between TTV and the host immune system is an active area of investigation. Overall, understanding the roles of TTV, hepatitis infections, and their links to liver diseases and HCC is essential for improving diagnostics, developing more effective treatments, and implementing preventive measures. As research continues, it is hoped that advances in these areas will lead to better outcomes for individuals affected by these infections and associated liver diseases.

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The Most Important Immunophenotypic Markers in Flow Cytometry

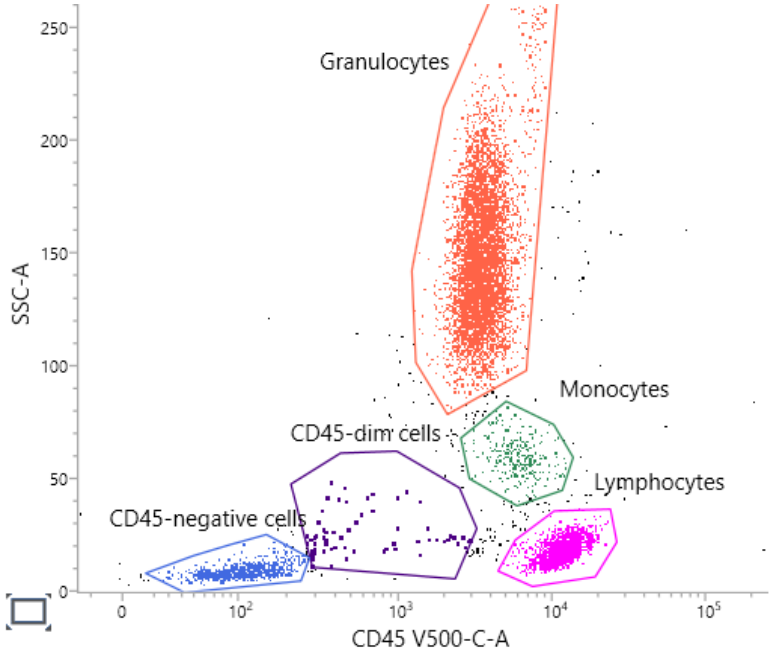
Alpaslan OZTURK

In this chapter, eleven immune markers that are most frequently used in the diagnosis of hematological neoplasia and diseases and can be analyzed by flow cytometry will be summarized. These markers are usually labelled with the abbreviation CD followed by numbers. CD stands for cluster of differentiation. It refers to molecules expressed on tumour cells or normal cells. Since there are specific markers for each cell, it is used as a tumour marker in practice. It shows which cell or lineage origin of haematological neoplasms.

CD45 (LCA)

CD45 is a receptor protein tyrosine phosphatase and is also called leukocyte common antigen (1). CD45 is expressed by all nucleated hematopoietic cells and their precursors. However, mature erythrocytes and platelets are CD45 negative cells (2,3). This molecule is also found in fibrocytes in peripheral blood, which have properties similar to those of connective tissue cells (4,5). CD45 has various isoforms including RA, RB and RC (6-9). Expression of these isoforms varies according to maturation, activation and differentiation status of cells. CD45RO is the smallest isoform (10,11). CD45RA molecule is positive in naive T lymphocytes. With activation, CD45RA is replaced by CD45RO (12,13).

Since CD45 is the common antigen of all leukocytes, SSC-A and CD45 dot plot is one of the first graphs analysed in flow cytometry analyses. It is shown in figure 1 below.



CD45 expression is weak in chronic lymphocytic leukaemia (CLL) cells (14). CD45 negativity has been found in Hodgkin lymphoma, a tumour of Reed-Sternberg cells, and acute lymphoblastic leukaemia (ALL) (15,16). CD45 molecule has a significant effect on prognosis

in ALL and multiple myeloma (MM). High CD45 expression is a poor prognostic factor for ALL (17). However, the presence of CD45 is a good prognostic factor for MM (18,19).

MPO

Myeloperoxidase (MPO) is the most specific immune marker for acute myeloid leukaemia (AML). This molecule distinguishes AML from ALL with a definite line (20). However, there are also AML types that can be MPO negative. AML types with MPO negativity are minimally differentiated acute myeloid leukaemia, AML with and without maturation, AML with NPM1 mutation, acute monoblastic leukaemia, acute basophilic leukaemia, acute mast cell leukaemia and acute megakaryoblastic leukaemia (21, 22).

CD33

CD13 is expressed in cell types developing from myeloid series (23). CD13 and CD33 molecules which are pan-myeloid antigens are positive in AML group. However, they are positive in 10-20% of ALL patients. With the development of monoclonal antibodies against CD33, CD33 analysis in flow cytometry has become much more valuable. In addition to AML types, CD33 can also be observed positively in B-cell-derived neoplasms such as CLL, albeit less frequently (24).

CD117 (c-KIT)

CD117 molecule is also called c-kit or kit (25). CD117 plays a role in many cellular events such as cellular signal transduction, metabolism, cellular growth, apoptosis, proliferation, migration and differentiation (26,27). CD117 positivity is observed in many hematological neoplasms, especially in myeloid series tumours (28,29).

CD117 is an immune marker with very high specificity in the diagnosis of acute myeloid leukaemia (30). Its presence in tumours such as MM indicates a good prognosis (31). CD117 is positive in many AML types including AML with and without maturation, acute myelomonocytic leukaemia, acute megakaryoblastic leukaemia and acute erythroid leukaemia. However, acute monoblastic leukaemia does not express CD117 (32).

TdT

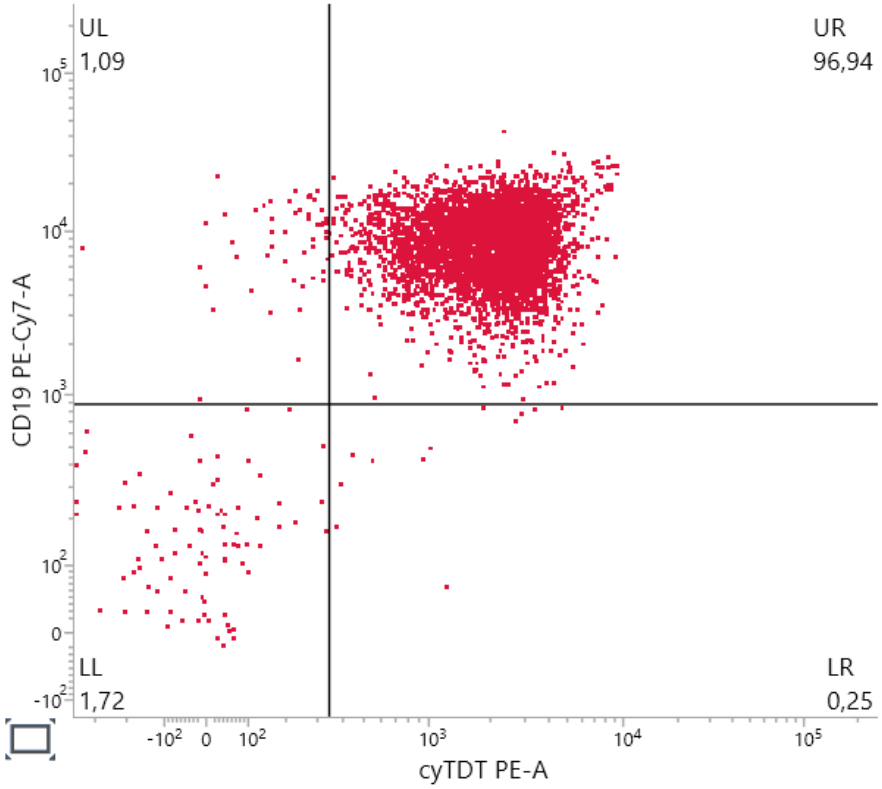
Terminal deoxynucleotidyl transferase (TdT) expression is seen in immature lymphoplasts. TdT positivity excludes leukaemia or lymphomas originating from mature cells. Nevertheless, TdT negativity is rarely observed in B-ALL cases (2%). TdT is a specific DNA polymerase enzyme found in immature pre-T and pre-B cells (33,34).TdT is positive in T-ALL, B-ALL, some subtypes of AML (especially minimally differentiated AML) and in immature T cells (thymocytes) in the thymus (35).

CD3

CD3 antigen is the most specific molecule for proliferations originating from T-cells. It is expressed in all T-cell-derived neoplasms, whether mature or immature. CD3 can be expressed in the cytoplasm and on the cell surface. In flow cytometric analysis of tumours developing from precursor T cells, surface CD3 is frequently negative (36). CD3 positivity may be observed in anaplastic large cell lymphoma, hepatosplenic T-cell lymphoma and enteropathy-associated T-cell lymphoma in addition to mature (peripheral) T-cell tumours (37,38).

CD19

CD19 is an integral membrane protein with a molecular weight of 95-kDa.CD19 expression is seen in B lymphocytes and follicular dendritic cells. CD19 positivity is detected in both immature and mature B cell-derived neoplasms. It is also an immunomarker that is positive in hematogon and some plasma cells. Figure 2 below shows the flow cytometric graph of TdT and CD19 positive B cell acute lymphoblastic leukaemia.



CD10

CD10 is a surface metalloendopeptidase with a molecular weight of 100 kDa. Another name of this molecule is common acute lymphoblastic leukaemia antigen (CALLA). Immature B and T cells, mature neutrophils, Burkitt lymphoma, follicular lymphoma and some types of diffuse large B-cell lymphoma are CD10 positive. Among precursor tumours, its positivity is much more common in B-ALL than in T-ALL. When samples of B-ALL patients are analysed by flow cytometry, very bright CD10 positivity is seen.

CD10 is also positive in hematogones which are benign B-cell precursors. However, CD10 is negative in myeloid and erythroid serial cell precursors. In mature neutrophils, CD10, like CD16, is brightly expressed. In myelodysplastic syndrome, the expression pattern of CD10 and CD16 may change and decrease (40,41).

CD5

CD5 is a pan-T marker with a molecular weight of 67 kDa. CD5 positivity is observed in immature and mature T cell-derived neoplasms. CD5, which should normally be present, is negative in benign T cells in conditions such as viral infections and bone marrow transplantation. CD5 positive benign B lymphocytes can be seen in peripheral blood and lymphoid tissues in children. Hepatosplenic T-cell lymphoma and enteropathy-associated T-cell lymphoma are CD5 negative. NK cells and NK cell tumours are CD5 negative. In B-cell

tumours, CD5 is characteristically positive in CLL and mantle cell lymphoma. CLL differs from mantle cell lymphoma with CD23 positivity (42,43). Follicular lymphoma is CD5 negative in contrast to CLL and mantle cell lymphoma. The tables given at the end of the chapter show the positivity and negativity of CD5 and other markers in B cell origin tumours (Table 1).

CD49d

CD49d (α4) has a molecular weight of 150 kDa. It forms the α4β1 integrin together with CD29 (β1). CD49d is expressed by B and T lymphocytes, thymocytes, Langerhans cells, eosinophils and monocytes (44,45). CD49d, an adhesion molecule, is also expressed on the surface of CLL cells. Many studies conducted and published in recent years have shown that CD49d elevation is associated with poor prognosis (45-48). CD49d is positive in approximately half of CLL cases and is one of the most important poor prognostic markers for CLL (45, 49).

CD34

CD34 is a small peptide molecule that binds to the membrane of pluripotent hematopoietic stem cells (HSC). The cell types that develop in CD34-positive HSC lineage are given in table 2 below. Early HSC, TdT-expressing immature lymphoid and immature myeloid cells are CD34 positive. This molecule can be detected both by flow cytometry and immunohistochemistry. CD34 is a positive marker in both ALL and AML.Among AML types, CD34 is frequently negative in hypergranular acute promyelocytic leukaemia and acute monoblastic leukaemia. However, CD34 is mostly positive in the hypogranular variant of acute promyelocytic leukaemia (50).

AML with NPM1 mutation is usually CD34 negative. AML types without NPM1 mutation but with FLT3-ITD are frequently CD34 and TdT positive (51).

Below are important tables related to immune markers analysed in flow cytometry. We think that these summary tables will be very useful in flow cytometry practice.

Table 1. Immunophenotype of mature B-cell lymphomas (52)

Neoplasia	**CD5**	**CD20**	**CD43**	**CD10**	**CD103**	**Surface Ig**	**Cyclin D1**
Follicular lymphoma	-	+	+	+	-	+	-
Chronic lymphoid leukemia	+	+	+	-	-	+	-
B-cell prolymphocytic leukemia	+	+	+	-	-	+	+
Mantle cell lymphoma	+	+	+	-	-	+	+
Splenic marginal zone lymphoma	-	+	-	-	-	+	-
Hairy cell leukemia	-	+	?	-	+	+	-

Table 2. *Hematopoietic stem cells-derived cells (53)*

CD34-positive HSC	
Common lymphoid progenitor cell	**Common myeloid progenitor cell**
• B cell • T cell • NK cell • Plasmacytoid dendritic cell	• Monocyte • Granulocyte • Basophil • Mast cell • Eosinophil • Red blood cells • Platelets • Monocytoid dendritic cell

Table 3. Important Immune Antigens and Associated Cell Types (54)

Antigen	**Associated cell type or neoplasia**
CD1	Thymocytes and Langerhans cells
CD3	Thymocytes, mature T cells
CD4	Helper T cells, subset of thymocytes
CD5	T cells and small subset of B cells
CD8	Cytotoxic T cells, subset of thymocytes, and some NK cells
CD10	Pre-B cells and germinal center B cells
CD19	Pre-B cells and mature B cells but not plasma cells
CD20	Pre-B cells after CD19 and mature B cells but not plasma cells
CD21	Mature B cells and follicular dendritic cells
CD23	Activated mature B cells
CD79a	Marrow pre-B cells and mature B cells
CD103	Hairy cell leukemias
CD25	Hairy cell leukemias
CD11c	Granulocytes, monocytes, macrophages, hairy cell leukemias
CD13	Immature and mature monocytes and granulocytes
CD14	Monocytes

CD15	Granulocytes; Reed-Sternberg cells and variants
CD33	Myeloid progenitors and monocytes
CD64	Mature myeloid cells
CD16	NK cells and granulocytes
CD56	NK cells and a subset of T cells, multiple myeloma
CD34	Pluripotent hematopoietic stem cells and progenitor cells of many lineages
CD30	Activated B cells, T cells, and monocytes; Reed-Sternberg cells and variants
CD45	All leukocytes; also known as leukocyte common antigen (LCA)

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The effect of heat-treatment on S-Allyl-L-cysteine (SAC) and antioxidant properties of Araban garlic

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Introductıon

Garlic (Allium sativum L.) belongs to the Liliaceae family, genus Allium, and is a plant that grows in soils rich in selenium and germanium. Throughout history, garlic has been used all over the world for various purposes, as a spice, vegetable or therapeutic. There are many vegetables that contain organosulphur compounds. Garlic (A. sativum), onion (Allium cepa) and leek (Allium ampeloprasum var. porrum) are known as alliaceous vegetables containing these compounds. Garlic is the most consumed and researched type of vegetable, mainly because of the organosulfur compounds it contains (21). Garlic is valued for its high antioxidant, anti-inflammatory, antibacterial and antifungal, immunomodulatory, antihypertensive, antihyperlipidemic, anticancer and hepatoprotective properties (1). The composition of fresh garlic includes carbohydrates (26-30%), proteins (1-1.5%), lipids (0.1-0.5%), organosulphur compounds (1.1-3.5%) and phenolic compounds (0.1-0.5%). Garlic is also a rich source of vitamin C, vitamin E, thiamine (B1), riboflavin (B2), niacin, calcium, sodium and minerals (iron, germanium and selenium).

The most common cooking methods used to prepare vegetables for consumption are boiling, steaming, frying, pressure cooking and microwaving. During cooking, the composition of vegetables changes, some compounds are broken down and others may be formed. Knowledge of the changes that occur both during cooking and during post-cooking processes such as waiting/storage is necessary to minimize deterioration and loss of nutrients. When applying heat treatment to foods for cooking, it is very important to choose the most appropriate cooking methods to preserve nutrients and obtain the best sensory properties. In this study, the effects of different heat treatments used to prepare foods for consumption on the bioactive properties of garlic were investigated.

Material and Method

Material

The fresh garlic used as material was obtained from the Araban district of Gaziantep province, which is located in the westernmost part of the Southeastern Anatolia Region and partly in the Mediterranean Region of Turkey. Garlic samples were subjected to two different heat treatments such as boiling and steaming for 1 and 3 minutes each. The antioxidant properties and SAC contents of both processed and fresh samples were determined.

Preparation of samples

Fresh garlic samples were cleaned from inedible parts in the laboratory and passed through pure water and then subjected to blanching and steaming.

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Boiling

Pure water (1:3 w/w) was used to boil the garlic. Pure water was heated to 100 °C using a steel pot and then garlic samples (250±0.1 g each) were placed in the pot and allowed to cool after 1 and 3 minutes of boiling.

Steaming

A stainless-steel steam cooking apparatus was used to steam the garlic. While pure water was boiling at 100 °C, the steaming apparatus was placed in a way to prevent the garlic from contacting the water and the garlic samples, each 250±0.1 g, were allowed to cool after 1 and 3 minutes of steaming.

Methods

Preparation of Extracts

For extraction, fresh and processed garlic samples were dried in a lyophilizer at -86 °C (FDU-8612, OPERON). The dried samples were then ground in a mortar and pestle. 4 g of the powdered samples were weighed into a beaker and 40 mL of acidified methanol (40 µL 37% HCl) was added. The samples were kept in an ultrasonic water bath for 30 minutes and centrifuged at 6000 rpm for 15 minutes in a centrifuge cooled to +4°C. The centrifuged samples were filtered through Whatman No. 42 and samples at a concentration of 100 mg/mL were prepared and used for total phenolic and antioxidant analysis. The remaining samples were placed in Petri dishes and stored in the refrigerator at -18 °C (2014 ND, Arçelik). Extraction for SAC analysis was performed according to Malaphong et al. 60 g of garlic samples were taken and thoroughly homogenized using Ultraturrax, then 1 g of the homogenized garlic samples was taken, 40 mL of distilled water was added and shaken for 1 min. It was then filtered through a 0.45 µm syringe.

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu method, a simple and widely used method based on electron transfer from phenolic compounds to FCR (Folin-Ciocalteu reducing capacity) in alkaline medium (8). Total phenolic content was determined according to Binici et al. (2) and Singleton and Rossi (20) modified Briefly, 100 µL sample of fresh and processed garlic extracts, 800 µL distilled water, 800 µL 0.5 N FCR, 800 µL 10% NaCO3 were added and vortexed. After 30 min. in the dark, it was measured in a spectrophotometer (PG Instruments T60V) at 760 nm wavelength by zeroing against the blind. 900 µL distilled water, 800 µL 0.5 N FCR and 800 µL 10% NaCO3 were used as blinds. Total phenolic content of the samples was calculated as mg GAE/g sample using different concentrations (10-250 µg/mL) of gallic acid solution.

DPPH˙ radical scavenging activity

DPPH analysis was performed according to Binici et al. (2), modified. 39 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl, 1 mM) was weighed and made up to 100 mL with methanol and kept in the dark for 12-16 h using a magnetic stirrer and wrapped in foil. Fresh and processed garlic samples (500-1000 µL) were transferred to test tubes. 500 µL of 1 mM DPPH and 2500 µL of methanol were added to the final volume and vortexed. Absorbance was measured at 517 nm against a blank (methanol) after 30 minutes in the dark. 2500 µL of 1 mM DPPH solution was used as a control. The equation was calculated as % inhibition. The IC50 value (mg/mL) was calculated from the % inhibition curve.

$ABTS^+$ analysis (2,2-azinobis(3-etilbenzothiazollin-6-sulfonik asit)

ABTS analysis was performed according to Re et al. (25) and Güzel and Akpınar (26) with modifications. A 7 mM ABTS solution containing 2.5 mM potassium persulfate was prepared. 0.0384 g of ABTS was weighed, dissolved in some distilled water and transferred to a 10 mL flask. 12.25 mM potassium persulfate solution was prepared in a separate flask. 0.3311 g of potassium persulfate was weighed and dissolved in some distilled water and the final volume was made up to 100 mL. The prepared potassium persulfate solution was taken up to 2 mL and transferred to ABTS solution. The final volume was made up to 10 mL with distilled water and 7 mM ABTS containing 2.45 mM potassium persulfate was prepared. This solution was kept in the dark for 12-16 hours to form the ABTS radical solution. The prepared solution was diluted with distilled water and the absorbance was adjusted to 0.700±0.02 at 734 nm. Fresh and processed samples (50-150 µL) were collected and transferred to test tubes. ABTS solution was added to 2500 µL. After 6 minutes in the dark, readings were taken against the blank (distilled water) at 734 nm. 2500 µL ABTS solution was used as control. The equation was calculated as % inhibition. The IC50 value (mg/mL) was calculated from the % inhibition curve.

SAC analysis by HPLC

Derivatization for SAC analysis was performed according to Malaphong et al. (13). Briefly, 250 µL of 10 mmol/L dansyl chloride and 650 µL of 20 mmol/L sodium tetraborate (pH 9.2) buffer were added to 100 µL of sample, vortexed, and allowed to derivatize for 15 min. The derivatized samples were then transferred to vials using a 0.45 µm nylon syringe and injected into the HPLC-UV system. A new method was developed for the analysis of SAC by HPLC using an Ace C 18 column (5 µm, 150 x 4.6 mm), 50 mM sodium acetate buffer (pH=5)-methanol (35:65, v/v) mobile phase, flow rate 1 ml/min, injection volume 10 µl, and 250 nm wavelength parameters. The retention time of SAC was recorded as Rt=5.8 min.

Statistical analysis

Analyses of variance were performed using SPSS 25.0 (SPSS Inc.). The means of statistically significant sources of variance were compared using the Duncan multiple comparison method. PCA analysis SIMCA-p+14.1, program UMETRICS, was used to determine the difference between samples.

Results and Discussion

The TPC, DPPH and ABTS+ values of fresh garlic samples are shown in Table 1. The TPC, DPPH (IC50) and ABTS (IC50) values of fresh garlic samples were determined to be 16.88 mg GAE/g, 23.88 mg/mL and 5.02 mg/mL, respectively. In a study by Kahyaoğlu (10), the total phenolic content and DPPH content of fresh garlic samples were determined to be 20.48-25.40 mg GAE/g and 122.82-146.52 mg TE/g, respectively. Chen et al. (4) determined the amount of total phenolics in fresh garlic between 10.17-22.66 mg GAE/g. Elosta et al. (6) found the amount of DPPH (IC50) in fresh garlic to be 24 mg/mL in fresh garlic in a study they conducted. It can be seen that our study is compatible with the literature.

Table 1. *TPC,DPPH,ABTS and SAC values of fresh and heat-treated garlic*

Processing	Times (minute)	TPC mg GAE/g	DPPH (IC_{50} mg/mL)	ABTS (IC_{50} mg/mL)	SAC (µg/g)
Fresh	0	16.88±0.37a	23.88±0.15e	5.02±0.03d	11.98±0.24a
Boiled	1	3.48±0.1d	129.97±1.56b	7.50±0.17a	6.24±0.19d
Boiled	3	2.58±0.13e	156.38±2.26a	7.03±0.09b	4.32±0.09e
Steamed	1	7.58±0.21c	55.12±0.12c	6.44±0.12c	7.08±0.21c
Steamed	3	13.14±0.10b	33.61±0.14d	4.02±0.04e	10.10±0.10b
Sig.		**	**	**	**

Different letters (a-d) in the same column are significantly different ($P < 0.05$) Abbreviations: SD: standard deviation; **: $P < 0.01$.

It was observed that the amount of total phenolic substances decreased in boiling and steaming garlic samples compared to fresh garlic and there was a statistically significant ($p<0.01$) difference. It was observed that the total phenolic content decreased in boiling garlic samples depending on the time. It is believed that the decrease in phenolic content is due to changes in matrix component due to deterioration of tissue structure and leakage (24). In the steaming garlic samples, the highest total phenolic content was found in the sample cooked in steaming for 3 minute and it was observed that the values increased with time. While the decrease in total phenolic content is associated with the degradation of phenolic substances as a result of heat treatment, the increase is attributed to the increase in free flavonols as a result of heat treatment (27). The inhibition concentration (IC50) is defined as the concentration of a substance required to reduce a biological process by half. Antioxidant activity is given by the IC50 value, which expresses the amount of antioxidant required to reduce 50% of the initial DPPH concentration. IC50 and antioxidant activity are inversely proportional. In other words, a lower IC50 value indicates higher antioxidant activity (2). The DPPH (IC50) values of boiling samples were between 129.97-156.38 mg/mL and the antioxidant activity decreased with time. The DPPH (IC50) values of steamed garlic samples were between 55.12-33.61 mg/mL and the antioxidant activity increased depending on the time. It was found that the highest antioxidant activity of the treated garlic was in the samples cooked in steaming for 3 minutes. The ABTS (IC50) values of boiling and steamed samples were found to be between 4.02-7.50 mg/mL and statistically significant ($p<0.01$). It was observed that the ABTS IC50 value of the garlic sample cooked in steam for 3 minutes was 4.02 mg/mL and showed higher antioxidant activity compared to fresh and processed garlic. Thus, it can be seen that the antioxidant activity of DPPH (IC50) and ABTS (IC50) values in cooked garlic decreased as a function of time. This decrease in antioxidant activity is thought to be due to losses due to degradation of the components that make up the antioxidant activity. Mehmood and Zeb (14) reported in a study that antioxidant degradation may be due to the transition to water during boiling and may affect antioxidant activity. DPPH and ABTS levels of steamed garlic were found to increase with time. In a study conducted by Xu et al. (2014), it was reported that there was no significant loss of DPPH values in steaming samples, while Murador et al. (15) reported that there was a significant increase in antioxidant activities of steamed samples. It was observed that the amount of SAC decreased in boiling and steaming garlic samples compared to fresh and there was

a statistically significant ($p<0.01$) difference. It is seen that the amount of SAC decreased in boiling garlic samples depending on the time. In steaming garlic samples, the highest amount of SAC was found in the sample cooked in steam for 3 minutes and it was observed that the values increased depending on the time. It is known that SAC has positive effects on human health (anti-inflammatory, anti-obesity, anti-apoptotic, cardioprotective, hepatoprotective, neuroprotective and antioxidant effects) and it is said that the amount of SAC in fresh garlic is 19.61 µg/g (21). The reason for the decrease in the amount of SAC in garlic subjected to boiling process; since the SAC compound is a water-soluble substance, it is thought that it may be due to the possibility of remaining in water.

Principal Component Analysis (PCA)

As seen in the correlation table (Figure 1), a very significant ($p<0.01$, $r2=0.981$) positive correlation was found between total phenolics and SAC, while a very significant ($p<0.01$, $r2=-0.925$; -0.876) negative correlation was observed between DPPH and ABTS. In other words, it was understood that SAC levels increased while total phenolic content increased and DPPH and ABTS levels decreased. In a study conducted by Zor et al. (24), it was reported that total phenolic content and DPPH and ABTS content were negatively correlated ($p<0.01$), $r2= -0.55$; $r2=-0.48$**). Therefore, it is believed that the amount of phenolic substances affects the antioxidant activity in the samples (3). Principal component analysis (PCA) was applied to determine the differences among the samples by evaluating the antioxidant activities, total phenolic content, and SAC content of raw and cooked vegetables prepared using two different cooking methods. Figure 2 shows the score scatter plot, loading scatter plot and biplot of the principal component analysis for raw samples and samples cooked by different cooking methods. The first two principal components (PC1 = 92.2% and PC2 = 4.84%) explained 97.04% of the variance.

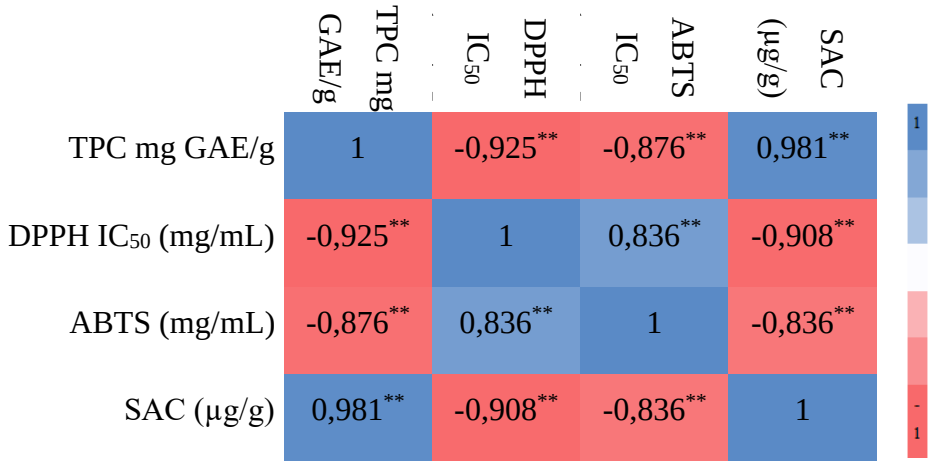
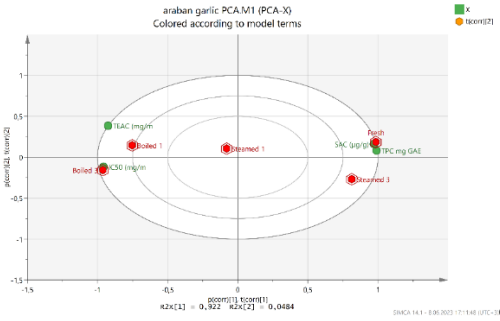
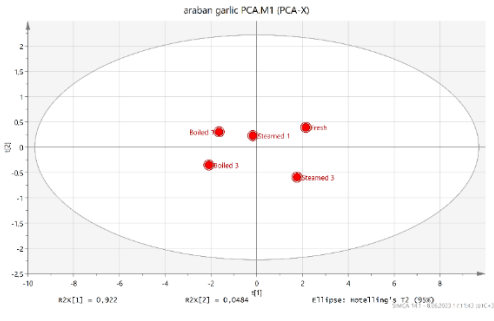


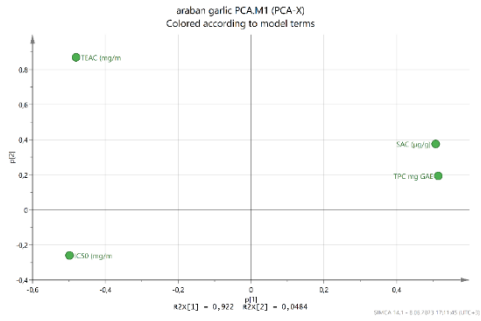
Figure 1. *TPC,DPPH,ABTS and SAC correlation values of fresh and heat-treated garlic*



a)



b)



c)

Figure 2. *Score plot (a), loading ccotter plot (b) and biplot (c) of principal component analysis of fresh and heat-treated garlic*

Conclusion

This study showed that steaming caused the lower losses in garlic and preserved TFM, antioxidant activity and SAC. Garlic is of great importance for human health due to its sulfur compounds and compounds formed by their degradation. Garlic, which is rich in nutrients and characteristic sulfur compounds that are not found in most other vegetables, should be considered in different alternative processes to prevent losses that may occur with processing. In this direction, when used in formulations, garlic should be added at the end of heat treatment and cooked with the steam in the environment and its functional properties should be preserved in this form.

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Allogeneic Stem Cell Transplantation And New Treatment Modalities For The Treatment Of Acute Leukemia İn Children.

Erdem AK

ALL is the most common malignant tumor in children. In Europe, the incidence rate under 15 is 40 per million. Significant advances have been made in treating acute leukemia in the last two decades. Today, the survival rate of ALL children is around 85%. However, the success of conventional treatment in relapsed and refractory acute leukemia is limited. Success with intensive chemotherapy is around 50%. (1)

Allo-HSCT is a curative treatment for acute leukemia. Many publications have shown the superiority of HSCT over intensive chemotherapy (2).

Acute leukemia treatment protocols are developed according to risk and leukemia type. With minimal residual disease (MRD) development, relapse and risk monitoring have started to be done better. More specific treatment protocols are developed according to MRD results. HSCT is the best treatment for high-risk, relapsed, and resistant leukemia. The source of the stem cell (peripheral, cord blood, or bone marrow) varies according to the compatibility of the stem cell and the degree of kinship.

In stem cell transplantation, if the source is the person himself/herself, it is referred to as autologous stem cell transplantation. If the source is not the person himself/herself, it is referred to as allogeneic stem cell transplantation. Allogeneic stem cell transplantation is preferred for acute leukemia transplants in children.

Allogeneic Stem Cell Transplantation

MSD: transplants with fully matched stem cells from parents or siblings as the source.

MUD: transplants with 8/8 or 7/8 HLA-matched stem cells from outside the family as the source.

Haploidentical: transplants from stem cells other than 8/8 and 7/8 HLA-matched stem cells within the family as the source.

Umbilical Cord: transplants with HLA-matched stem cells in cord blood as the source.

Donor Selection:

The primary choice of donor for stem cell transplantation in ALL is an HLA-matched sibling donor. In recent years, unrelated, full matched donors are also preferred. Studies on overall survival (OS) between matched related and unrelated matched donors have yielded similar results. (3,4) Unrelated matched donors of the same race and ethnic group are similar. Men under 40 years of age are preferred in donor selection in transplantation. (5)

Recently, haploidentical donor selection and transplants have been increasing and developing. The use of tacrolimus, cyclophosphamide, and mycophenolate after transplantation has led to a decrease in the rate of acute and chronic graft versus host disease (GVHD) (6) Similar results have been reported in studies comparing OS in unrelated full-matched (MUD) and haploidentical transplantation (7).

Haploidentical HSCT donors are easier to access than unrelated full matched donors. In a retrospective study of 822 AML patients, Penoleas et al. examined 192 haploidentical and 631 MUD transplant patients between 18 and 60. Both groups received a myeloablative preparation regimen. In haplo HSCT, cyclophosphamide was given post-transplantation. 5-year overall survival was 32% in haplo- HSCT and 42% in MUD transplants. (8) The most significant disadvantage of this study is the difference in numbers between the study groups. The relapse rate was found to be lower in transplants performed with unrelated donors.

Advantages and disadvantages between haploidentical and MUD HSCT:

- It is easier to find haploidentical donors.

- In haploidentical transplantation, transplantation can be performed rapidly after family typing

- The risk of hemorrhagic cystitis and cardiogenic toxicity due to post-transplantation cyclophosphamide is higher.

- Hematologic recovery time is longer in haploidentical transplants

- The risk of acute and chronic GVHD is lower in haploidentical transplantation due to post-transplantation cyclophosphamide.

- Depending on race/ethnicity, it may be difficult to find HLA-matched donors.

- Preparation regimens similar to those in matched related transplants are applied.

- It is possible to get rid of donor-specific anti-HLA antibodies

In haploidentical transplants, hemorrhagic cystitis is the most common complication due to post-transplantation cyclophosphamide. Other essential complications are cardiac toxicity and renal failure. Post-transplant cyclophosphamide causes a delay in engraftment and hematologic recovery. However, the risk of GVHD increases. (9) If there is no unrelated matched or cord donor, haploidentical transplantation is the only transplantation option. Cousins or other 2nd-degree relatives can be used as donors in haplo-HSCT. Anti-HLA antibodies are one of the biggest problems in haploidentical transplants. If anti-HLA antibody positivity is present, the risk of graft failure is very high. Sometimes it may not be possible to find anti-HLA-positive donors. HLA-DP may be incompatible if HLA A, B, C, and DQ are compatible in unrelated matched donors. The presence of anti-DP antibodies may cause graft failure. (10)

When transplants with matched sibling donors (MSD) and 8/8 HLA unrelated full matched (MUD) donors were compared with transplants with 7/8 matched MUD donors, OS was lower and acute and chronic GVHD were higher in transplants with 7/8 HLA matched donors (11). In a retrospective study conducted by EBMT, haploidentical transplants using post-transplantation cyclophosphamide were compared with MUD and MSD lymphoma and leukemia transplants. Non-related mortality (NRM) was higher in 7/8 HLA-matched MUD and MMUCD (mismatched umbilical cord) transplants than haploidentical transplants. (12) Transplants with haploidentical donors should be preferred over transplants with mismatched unrelated donors. The risk of relapse is lower in 7/8 HLA-matched transplants. However, when analyzed regarding EFS (event-free survival), no significant difference was found between both groups. Transplants with 7/8 MUD and MMUCD donors have lower overall survival than transplants with haploidentical donors. (13)

In donor selection, if the patient's CMV Ig is positive, CMV positive donor should be preferred. If the patient's CMV Ig G is negative, CMV Ig G negative donors should be preferred.

In donor selection, young patients should be preferred if enough CD34+ positive cells can be collected.

Prognostic factors and Indications in ALL:

Pediatric acute leukemia generally has a good prognosis. Stem cell transplantation is indicated in high-risk patients, leukemias not responding to pretreatment and relapsed patients. With the development of minimal residual disease (MRD), the indication criteria for HSCT have become more detailed. High-risk patients with unfavorable cytogenetics have a higher risk of relapse after complete remission. CAR T cell therapy and immunotherapy have been considered as alternative therapies in high-risk patients. CAR T cell therapy or immunotherapy can be used as bridging therapy before transplantation to turn MRD negative in highly refractory ALL. (14)

In newly diagnosed B-cell lymphoma patients, unfavorable genetics and positive MRD after induction are indications for HSCT. Hypodiploidy and Philadelphia chromosome positivity (Ph +) in CR 1 (complete remission 1) are also indications for HSCT. According to the St. Jude Total ALL study, chemotherapy alone and HSCT + chemotherapy treatments were compared in terms of overall survival in hypodiploidy MRD-positive patients, and 5-year OS was found to be 41% to 85% (15).

In Ph+ ALL patients, a 5-year OS of 80% was achieved with combined treatment of tyrosine kinase inhibitor and chemotherapy. Persistent MRD positivity in Ph+ ALL patients indicate HSCT (16). In standard risk patients, there is an indication for stem cell transplantation with MRD positivity values between 0.1% and <1% after the consolidation treatment. However, in the latest revised treatment protocols of COG (Children Oncology Group) and NCI (National Cancer Institute), blinatumomab was added in the last two cycles.

Primary induction failure: the presence of leukemic blasts in the bone marrow (M2 marrow (blasts 5%-25%), M3 marrow (blasts >25%)) or extra nodular involvement after induction therapy.Poor prednisone response: peripheral blood blast count above 1000 blast cells/uL on day eight after prednisone monotherapy. According to the ALL-BFM protocol, MRD is considered high risk when blasts are detected above 10% on day 15 of induction therapy (except ETV6-RUNX 1, E2A-PBX, and KMT2A). After PCR evaluation of leukemia-specific immunological markers after consolidation, MRD level is considered high risk if it is detected above 5×10^4.After induction therapy (EOI), PCR-MRD is considered high risk when detected above 10^3.

One study comparing the overall survival of patients who received HSCT treatment under six months and patients who received only intensive chemotherapy, 10-year OS was 72% and 59%. (17) EsPh-ALL protocol has been given for Ph+ acute leukemia since 2004. Early and long-term imatinib treatment is available in this protocol (18). According to the EsPh ALL protocol, EOC-MRD above 5x10 4 indicates transplantation.

High Risk ALL Genetic Mutations:

- t (9,22) BCR-ABL

- t (4,11) KMT2A-AFF1 (MLL-AF4)

- t (17,19) (TCF-HLF)

- IKZF

High-Risk Groups According to AIBOP-BFM 2009:

- KMT2A-AFF1

- Low Hypodiploidy
- MRD positivity on flow cytometry (d15)
- Induction Failure
- PCR-MRD positivity (EOC time)

HLA MSD Transplant Indications:

- PCR-MRD positive between 10^3-10^2 (after consolidation (BOC time))
- KMT2A-AFF1 positivity

HLA Unrelated MMD Indication

- Induction failure
- PCR-MRD positive over 10^2 (BOC time)

Indications for HSCT according to the AIBOP-BFM 2017 Study:

- TLF3-HLF gene fusion
- KMT2A-AFF1 gene fusion
- MRD positivity in IKZF1 plus deletion (at d15)
- PCR-MRD positivity

European ALL Together 1 Indications for HSCT:

- MRD>= 0.05% (EOC time) (TP1)
- MRD>= 5% (EOI time) (TP2)
- MRD>=0.5% (Mid-consolidation time, d50)
- MRD positivity in t(17,19) (q22p13) TLCF3/HLF mutation, TP1, TP1,5 or TP2
- Detection of MRD above 0.05% at the time of TP2 in patients with leukemia in the high-risk group.

In the past years, treatment of T-cell ALL was complicated. OS and EFS were low compared to B-cell ALL. Today, with the intensification of treatment and the addition of new drugs, the success of OS and EFS has become closer to B-cell ALL. MRD determines risk classification in cell leukemia after induction and consolidation. (19,20)

High-risk groups for T-cell ALL according to AOBOP-BFM:

- Prednisone poor response
- FCM-MRD positivity (d15th day)
- PCR-MRD positivity above 5x10/-4 on days d33 and d78 (TP2).
- PCR-MRD level > 10/-3, especially during EOC. (21)

Indications for HSCT in Complete Remission 1 in T Cell Leukemia:

- MRD >== 5% (at the time of TPI)
- MRD>= >0.5% (at time TP1.5), >1% (at time TP2)

- PCR-MRD level <5% in TP1 and above 0.05% in TP2.
- Primary induction failure

ALL risk stratification at the first relapse

BFM group:

Low(S1);

- Late IEM relapse
- Early IEM relapse (isolated extramedullary relapse)
- Late B ALL isolated bone marrow relapse.
- Early B ALL combined relapse

High Risk

- Very early and early B ALL bone marrow relapse.
- Very Early B ALL combined relapse.
- T-ALL bone marrow relapse (time-independent)

Very Early Relapse: Relapse 18 months before diagnosis.

Early Relapse: Relapse 18 months after diagnosis but six months before the end of treatment.

Late Relapse: Relapse 6 months after the end of treatment.

COG (Children Oncology Group) First relapse risk classification:

Low-risk group:

- Late B-ALL bone marrow relapse (block 1) MRD 0.1% <
- Late IEM end-block MRD 0.1% <

Intermediate risk group:

Late B ALL relapsed bone marrow involvement (MRD > 0.1% in end-block 1)

High Risk Group:

- Early B-ALL bone marrow involvement
- Early IEM
- T-ALL relapse (at any time)
- KMT2A rearrangement, 6 mo. < WBC > 300000 (at diagnosis)
- Ph-positive ALL:
- Prednisolone poor response
- MRD >= $5x10^4$ (at EOC)
- MRD<5X10/-4 and positive MRD at the end of the HR3 block.
- ALL in Down syndrome

- MRD>= 0,01 (at EOI)

- MRD>=0.5% (double trisomy 4 and 10 group)

There is a phase II study on blinatumomab in ALL patients with intermediate and high-risk Down syndrome (23).

- Mixed Phenotypic ALL

- Mixed phenotypic ALL is generally considered high risk. AML chemotherapy protocols are usually applied, and HSCT is not needed. (24)

- Indications for HSCT according to COG protocol in mixed type:

- MRD > 5% (at EOI)

-MRD>0.01(at EOC)

HLA Compatibility	**HLA identical sibling donor**	**Other family donor**	**Unrelated Donor**
10/10	MSD	MD	MD
9/10	-	MD	MD
Less than 9/10	-	MMD	MMD

Table.1: Donor typing; MSD: Matched sibling donor, MD: Matched donor, MMD: Mismatched Donor

MSD (matched sibling donor)	HLA matched identical sibling
MD (Matched Donor)	5/6 or 6/6 HLA matching
MMD (Mismatched Donor)	Less than 5/6 HLA matching

Table. 2: Umbilical Cord Donor Typing

CONDITIONING REGIMES

TBI ALL REGIMENS (25,26)

Functional TBI (12 Gy given 2 Gy x2 at -7, -6,5 days)

- Etoposide (VP16); 60 mg/kg (max: 3600 mg) (-4.day)

BUSULFAN-CYCLOPHOSPHAMIDE ALL regimen (Patient >2 years old)

- It (Busulfan) iv/po (-7, -6, -5, -4. Days) (16 doses)

- Cyclophosphamide iv (-3, -2. days) 60 mg/kg/dose

Patient >2 years old:

- Bu (Busulfan) iv/po (-7, -6, -5, -4. Days) (16 doses)

- Cyclophosphamide iv (-3, -2. days) 60 mg/kg/dose

- Melphalan 140 mg/m2 (-1 days)

GVHD prophylaxis;

- Cyclophosphamide 3 mg/kg/day

-Methotrexate +1, +3,6 days

-ATG (Frenisusimus) 20 mg/kg/dose (-3,2, -1 days)

Myeloid engraftment: Absolut neutrophil count above 0.5 x 10^9 for three consecutive days.

Platelet aggression: Thrombocyte count above 50 x 10^9 for seven consecutive days.

In MSD transplants in CR1, EFS is 81%, 69% significantly higher than unrelated MD. However, overall, a survivor is the same. NRM is lower in MSD than in MD (7% versus 17%) (27). GVHD prophylaxis is different in MSD and MD. Mtx and CSA are given in MD. In MSDs, only CSA (cyclosporin) prophylaxis is used. (27) The incidence of cGVHD is lower in MSD than in MD (10% versus 36%). The incidence of aGVHD is also lower in MSD than in MD (22% versus 46%) (28). Under 12 years of age, the risk of CGVHD is higher than in children over 12 years of age. Above 12 years of age, the use of more peripheral blood donors in this group is an essential factor in the incidence of CGVHD. The risk of cGVHD increases significantly in patients using peripheral blood donors. However, no significant difference was detected in terms of EFS and OS. (29)

Haploidentical transplants are important alternatives in patients without MSD and MD donors. DLI (Donor lymphocyte infusion) can be performed in haploidentical donors and has a higher GVL effect.

Haploidentical HSCT Preparation Regimens:

- Cytarabine 4 mg/m2/day (-10, -9th Days)

- Busulfan (3.2 mg/kg/day) iv (-8, -7, -6, -5)

- Cyclophosphamide (1.8 mg/m2/day) (-5, -6 days)

- Senmustine 250 mg/m2 (-3 days)

- ATG (Thyroglobulin; Rabbit ATG) -2.5 mg/m2/day (-5, -4, -3, -2)

- Cyclosporin 2.5 mg/m2 q12h iv (-1 - +150 days)

- Mycophenolate Mofetil 0.25 -0.5 g po (+1-60 days)

- Short-term Mtx 15 mg/m2 (+1, +3, +6, +11 days)

- G-CSF (+6. Days starts) (5 ug/kg/d) (discontinued when white blood cell count is above 2 x 10^9 for three consecutive days)

UCBT HSCT Preparation Regimen:

- TBI (Total 12 Gy 4 fractions)

- Cy 60 mg /kg/day (2 days)

- Busulfan (3,2 mg/kg/day) iv (-8, -7, -6, -5)

- Cyclophosphamide (1.8 mg/m2/day) (-5, -6 days)

- Cytarabine 4 mg/m2/day (-10, -9th Days)

- Fludarabine 30 mg/m2 (4 days)

- Cyclosporin 3 mg/m2 q12h iv (-1 - +30 days) (Target level: 200-400 mg/ml

- Mycophenolate Mofetil 0.25 -0.5 g po (+1-30 days)

- G-CSF (+6. Days starts) (5 ug/kg/d)

Pneumocytes carinii, HSV, and fungal prophylaxis are given. There is no CMV prophylaxis. CMV treatment is given according to CMV-DNA level. Minimal Residual Disease (MRD) is routinely checked from bone marrow at 1,2,3,4,4,5,5,6,9 and 12 months. If GVHD cannot be controlled or a life-threatening infection is present, DLI treatment is indicated. Immunosuppressive treatment should be discontinued rapidly after the diagnosis of hematologic relapse, regardless of the duration of transplantation.

In cord blood donor selection, HLA-A, HLA-B, and HLA-DR loci should be identified in all patients. It is recommended that HLA-A and HLA-B should be screened with an intermediate DNA resolution technique, and HLA-DR1 should be screened with a high-resolution DNA technique. Cord Blood donors should have 4/6 or more matches. Cord blood is given at the level of 3 x 10^7 nucleated cells/kg or 1.2 x 10/5 CD34+. (29)

The cumulative incidence of grade III-IV aGVHD after day +100 was found to be (33.8% vs 48.9%) in haploidentical and UCBT transplants, respectively. 2-year cumulative incidence

of relapse was found to be (16.1% vs 24.1%) in haploidentical and UCBT transplants, respectively. (30)

Unmanipulated haploidentical and UCBT donors are alternative therapies in high-risk ALL patients when HLA MSD and unrelated full-matched donors are unavailable.

CD34+ count is higher in haploidentical donors compared to UCBT donors. Low CD34+ count leads to delayed hematologic recovery. 2-year DFS (disease-free survival) is much better in haploidentical HSCT than in UCBT.

HSCT is a practical, potentially curative treatment modality. However, the non-relapse mortality rate in patients undergoing TBI (total body irritation) is 20-40%. (31,32) The non-related mortality rate is low in patients undergoing RIC (reduced induced conditioning). However, the relapse rate is increased. Busulfan and melphalan regimen has lower toxicity compared to the TBI regimen. However, the risk of GVHD increases. The busulfan-melphalan regimen's non-related mortality rate is slightly higher (33).

TBI-free regimens are being tried to get rid of long-term toxicity. Regression in development and cognitive functions are the most important criteria. (34) Secondary malignancy, diabetes, cardiac toxicity, and atherosclerotic cardiovascular diseases are other side effects of the TBI preparation regimen. (35,36) Busulfan-Cyclophosphamide (Bu: 16 mg/kg Cy: 120 mg/kg) is an alternative preparation regimen. Busulfan-Fludarabine and Busulfan-cyclophosphamide are other alternative regimens.

Clofarabine is the agent used in relapsed and refractory ALL. The Clofarabine-Busulfan preparation regimen provides better results than the TBI regimen. (37)

Blinatumomab bispecific T cell (BITE) is an antibody that binds directly to CD19 and CD3 antigens. CD19 is expressed in precursor B cells and causes the activation of T cells. In malignant B cells, it causes apoptosis by T cells with a cytotoxic effect. It is approved by the FDA for use in CD19 ALL with MRD > 0.1% in CR1 and CR2. Bilitunamab is used as bridge therapy in HSCT in R/R ALL (39). Pre-transplantation administration decreases MRD. Studies have reported that it decreases EFS, toxicity, and OS. (40)

The most critical side effects of blinatumomab are neurotoxicity and cytokine release syndrome (CRS). Neurological/neuropsychiatric problems are the most common side effects (tremor, disability, somnolence, generalized seizure, encephalopathy)

In cord donor selection, HLA-A, HLA-B, HLA-C, and HLA-DR should be screened in all donors. It is recommended to screen HLA-A and HLA-B with the HLA-A and HLA-B intermediate resolution technique and HLA-DRBQ with the high DNA resolution technique. Cord donors should be matched 4/6 and above. 3 x 10 7 / kg or 1.2 x 10 5 CD 34 + / kg cord blood is required (29).

The incidence of severe CMV infection at post-transplant day 100 was (75% 64%) in haploidentical HSCT and UBCT donor transplants, respectively. The incidence of grade III-IV GVHD after day 00 was (33% and 48.9%) in haploidentical HSCT and UBCT donor transplants, respectively. (30) . The 2-year cumulative incidence of relapse was 16.1% in haploidentical transplants and 24.1% in umbilical cord graft transplants. (30)

Unmanipulated haploidentical and UBCT donors are alternative treatments for high-risk leukemias in patients with HLA MSD and unrelated full-match donors. CD34+ count is higher in haploidentical donors compared to UBCT. Low CD34+ count leads to delayed hematologic recovery. 2-year DFS (disease-free survival) is better in haploidentical transplants than in UBCT donor transplants. HSCT is a practical, potentially curative treatment. However, the TBI (total body irritation) non-relapse mortality rate varies between 20-40%. (31, 32)

With RIC (Reduced induced conditioning) the rate of acute NRM decreased, but the risk of relapse increased. Toxicity is less with the busulfan and melphalan regimen compared to the TBI regimen. However, the risk of GVHD increases. Non-related mortality was found to be lower in the busulfan-melphalan regimen. (33) TBI-free regimens are being tried to reduce long-term toxicity-related complications. The most important long-term complications of the TBI regimen are developmental and cognitive decline. (34) Secondary malignancy, cardiac toxicity, diabetes, and atherosclerotic cardiovascular diseases are other significant side effects. (35,36)

Busulfan-cyclophosphamide (Bu: 16 mg/kg Cy: 120 mg/kg) is another alternative preparation regimen. Busulfan-fludarabine and Bu-Cy are alternative regimens in ALL. Clofarabine is one of the agents that can be used in relapsed or refractory ALL. The clofarabine-busulfan preparation regimen is an alternative to the TBI regimen with good results (37).

Blinatumomab bispecific T cell engager (BITE) is an antibody that binds directly to CD19 and CD3 antigens. CD19 is expressed from precursor B cells and causes activation of T cells. It causes apoptosis by T cells with cytotoxic effect on malignant B cells (38) It was approved by the FDA in patients with relapsed or refractory CD19 ALL patients with CR1, CR2 with minimal residual disease > 0.01%. Blinatumomab is used in the first consolidation protocol for bridging therapy in HSCT in R/R ALL. (39) Using Blinatumomab before transplantation reduces the MRD+ rate and has been reported to reduce DFS toxicity rates and increase OS rates. (40) The most critical side effect of Blinatumomab is a neurotoxin and cytokine release syndrome (CRS). The most common neurological/neuropsychiatric side effects are tremors, somnolence, generalized seizure, encephalopathy, and dyskinesia.

T-ALL accounts for 15% of acute leukemias. T-ALL 5-year overall survival is 20% lower than B-ALL. (41,42) Nelarabine is an analog of deoxyguanosine (water-soluble ARA-G product) and has a toxic effect by causing DNA inhibition. The FDA approves Nelarabine in R/R T-ALL. Salvage therapy in R/R T-ALL is used as bridging therapy before transplantation.Nelarabine, CAR-T cell, inotuzumab ozogamicin and Blinatumomab are new, critical alternative therapies in salvage and bridging therapy before transplantation.

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