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# Haematological Profile of Rat Fibrosarcoma Models After Virulent Newcastle Disease Virus Virotherapy: A Pilot Study

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**ABSTRACT**

Virotherapy is the use of viruses as cancer therapy. One of the viruses that are declared to have oncolytic activity is Newcastle Disease Virus (NDV). In the development of virotherapy, there are fundamental things that need to be considered, one of which is the side effects caused. The haematological profile is one of the important indicators in assessing general health status. This pilot study aims to describe haematological profile of fibrosarcoma rat models after virotherapy with virulent NDV. This study used one-group pretest-posttest design, consisting of six white rats which were all induced fibrosarcoma with benzo(α)pyrene. After the tumour appeared, the rats were given a single treatment, i.e., virotherapy using NDV Tabanan-1/ARP/2017 isolate at a dose of 0.5 mL of 9 log 2 HA Unit. Blood sampling was performed on the orbital sinus, carried out at the following time points: before rat adaptation, after the fibrosarcoma induction, and after the therapy with NDV. The results of this study indicate that NDV Tabanan-1/ARP/2017 do not cause pathological changes on the rat haematological profile, and many values are better than cancer patients in general. Anemia and leukocytopenia that occur in general cancer patients, did not occur in rats in this study. The levels of RBC, Hb, PCV, WBC at the end of the study were as follows: 7.73±0.14 $x10^6$ µL; 12.8±0.42 g/dL; 39.5±3.53%; 10.7±5.09 $x10^3$ µL. Meanwhile, the differential leukocytes result for neutrophils, eosinophils, basophils, monocytes, and lymphocytes were as follows: 46.5±20.50%; 0.5±0.70%; 0; 5±0%; 48±19.79.%. From these results, it can be concluded that the NDV is a promising and has good potential as a virotherapy agent.

**Keywords**
haematological profile, rats, virotherapy, Newcastle disease virus, fibrosarcoma

## Introduction

Malignant tumours or commonly known as cancer is degenerative disease characterized by uncontrolled cell proliferation and has the potential to spread to other parts of the body (WHO, 2021). This disease causes serious problems in both animals and humans. Cancer not only causes pathological abnormalities locally but systemically. One of the changes that occur in cancer patients is the haematological profile. Patients with cancer will experience haematological disorders such as anemia, thrombocytopenia, thrombosis (Chauhan, 2010), neutrophilia, lymphopenia (Ménétrier-Cau *et al.*, 2019), and monocytosis.

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Significant developments in cancer therapy have been achieved in the last few decades, including accurate diagnosis of cancer types, cancer surgery, chemotherapy, and radiotherapy (Souto *et al.*, 2009). Surgery is a is quite good technique for cancer treatment because it does not have an effect on other normal cells, so immune cells can still work against tumour cells. However, surgery cannot be performed on several advanced stage cancer because it can increase the risk of metastasis (Tohme *et al.*, 2018). On the other hand, chemotherapy and radiotherapy are effective in killing cancer cells and clearing metastases but can cause damage to normal cells and cause changes in the body's physiological systems, such as the haematological profile (Takariyanti *et al.*, 2021). Chemotherapy is generally hematotoxic immunosuppressive so that patients who have experienced previous haematological changes due to cancer can experience complications. These complications can increase morbidity, even mortality (Miszczyk and Majewski, 2018).

Because of that, many researchers are trying to find alternative therapies that have minimum side effects. Today, innovation has emerged that utilizes viruses as cancer therapy (Kelly and Russel, 2007). The use of viruses as cancer therapy, also known as virotherapy, is a new treatment modality that uses viruses to selectively destroy cancer cells without damaging surrounding normal cells (Russel *et al.*, 2012). This idea arose accidentally in 1950 when patients with metastatic melanoma regressed after being injected with the rabies vaccine. In the following years, the development of research on virotherapy grew so rapidly (Kelly and Russel, 2007). To date, many viral strains have been declared to have oncolytic activity (Fountzilas *et al.*, 2017). One of the viruses that have oncolytic activity is Newcastle disease virus (NDV) (Sinkovics and Horvath, 2000; Sewoyo *et al.*, 2021b).

NDV is a very promising cancer virotherapy agent because it has diverse oncolytic activity (Jiang *et al.*, 2018). This virus is the cause of Newcastle disease in poultry which can cause huge economic losses. In poultry, the virulent strain of NDV is capable of causing severe damage and lesions to several internal organs (Adi *et al.*, 2009). In mammals, this virus can replicate slowly in normal cells (Schirrmacher, 2017). Several reports indicate that the NDV is only capable of causing mild flu in humans (Tayeb *et al.*, 2015). In contrast, in cancer cells, this virus is able to replicate 10,000 times faster than normal cells (Kalyanasundram *et al.*, 2018).

However, in order to be used as a therapeutic agent, research to test the safety of this virus is needed. The fundamental thing in the development of virus-based therapy is that the damage caused is more dominant in tumour cells than healthy cells. In addition, the side effects tend to be low, have a high replication rate, and can suppress tumour cell growth effectively (Vile *et al.*, 2002). One important indicator is the haematological profile. Haematological profiles are useful for assessing health conditions, diseases, and also the presence of tissue damage disorders (Ihedioha *et al.*, 2012). Based on this background, this pilot study aims to determine the haematological profile of tumour-bearing rats after treated with a virulent strain of NDV.

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## Material and Methods

This research has been officially approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University with approval number B/36/UN14.2.9/PT.01.04/2021. The procedures carried out in this study were in accordance with the provisions of the ethics committee. This study used six male white rats (*Rattus norvegicus*) Sprague-Dawley (SD) with an average age of three months with a body weight range of 120-160 g. All experimental animals were kept in Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University. Prior to the treatment stage, the animals were first adapted for one week. All rats were kept in the same environment and diet. During the seven-day adaptation process, the animals were treated humanely and given access to food and *ad libitum* water.

This research design is a one-group pretest-posttest design, which uses one group that is given the same treatment, then observed at three different time points. At these time points, blood was taken and collected. The first time point at $2^{nd}$ weeks, is the time point at which the rats were not given post-adaptation treatment. The second time point is $5^{th}$ weeks, after the induction of fibrosarcoma with benzo(α)pyrene. The fibrosarcoma induction refers to the procedure of Sewoyo *et al.* (2021a) using 0.3% benzo(α)pyrene dissolved in olive oil. The benzo(α)pyrene solution was injected ten times gradually at the interscapular area, with an interval of every two days. Week 20 was the time point when the rats had treated with the virulent NDV. The NDV isolate that were used was Tabanan-1/ARP/2017 (Adi *et al.*, 2019; Sewoyo *et al.*, 2021b). The dose given is 9 log 2 HA Unit/0.5 mL, administered for four days with one injection per day (Sewoyo *et al.*, 2021b).

Blood samples were taken in the orbital sinus area. Before taking blood, the rats were anesthetized using 10% ketamine HCL (Agrovet Market SA, Lima, Peru) intramuscularly at a dose of 50 mg/kg BW. Then the blood was taken aseptically using a microcapillary haematocrit, then collected into an ethylenediamine tetra-acetic acid (EDTA) tube. This tube is then put into a cool box until it is used for routine haematological examinations.

The collected blood samples were then examined with an auto haematological analyser (RT-7600®, Rayto Ltd., Shenzhen, China) at Laboratory of Parasitology of the Animal Disease Investigation Centre Denpasar. The analysis with these tools is carried out according to the instructions from the manufacturer. The variables tested included red blood cells (RBC), haemoglobin (Hb), packet cell volume (PCV) or haematocrit, white blood cells (WBC), and differential leukocytes. To calculate the leukocyte differential, manual calculations were performed on blood smear preparations that had been stained with Giemsa using a light microscope (Nikon, Tokyo, Japan). Haematological analysis data were presented in the form of mean ± SD (standard deviation) and analysed descriptively. The data were then also compared with the haematological profile of general cancer patients.

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## Results

The results of haematological profiles of rats before and after NDV therapy in each treatment are presented in Table 1. In general, RBC, Hb, and PCV have an important role in diagnosing disease, especially anemia (Thrall, 2004). In this study, these variables were still in the normal range, from the 2nd weeks until 20th weeks (Figures 1 and 2).

**Table 1.** The hematological profile of rats before and after NDV therapy

| Parameters | Week 2 | Week 5 | Week 20 | Reference range |
|---|---|---|---|---|
| RBC ($x10^6$ µL) | 9.18±1.16 | 7,13±0.13 | 7.73±0.14 | 7-11 |
| Hb (g/dL) | 14.96±1.89 | 11.6±0.43 | 12.8±0.42 | 11.6-16.1 |
| PCV (%) | 32.33±6.42 | 34.66±4.16 | 39.5±3.53 | 18-48 |
| WBC ($x10^3$ µL) | 8.83±1.70 | 8.36±1.79 | 10.7±5.09* | 2-10 |
| Neutrophils (%) | 33±25.51 | 28.66±8.50 | 46.5±20.50* | 12-38 |
| Eosinophils (%) | 1.33±1.52 | 0 | 0.5±0.70 | 0-6 |
| Basophils (%) | 0 | 0 | 0 | 0-2 |
| Monocytes (%) | 14±5.29* | 10.33±3.21* | 5±0 | 1-6 |
| Lymphocytes (%) | 55±24.26** | 57.33±5.50** | 48±19.79** | 60-75 |

Note: RBC=Red Blood Cells; Hb=Hemoglobin; PCV=Packed Cell Volume; WBC=White blood cell. *Above the reference range **below reference range

Based on these results, it can be concluded that the rats did not experience anemia in this study. Meanwhile, WBC was still in the normal range from the first to the 5th weeks, and in the 20th weeks it was above the normal value.

## Discussions

The results of the differential leukocytes at 2nd weeks showed that the rats had monocytes levels above the normal range, or monocytosis, while the levels of other cells were still in the normal range. The high levels of monocytes at the beginning of the treatment may be caused by stress in experimental animals. Stress can induce an increase in monocytes (Guereschi *et al.*, 2008).

At 5th weeks, there was a decrease in the value of RBC, Hb, WBC, and PCV. The decrease in this variable was caused by the tumorigenesis process that occurred after fibrosarcoma induction using the chemical carcinogen benzo(α)pyrene. The study conducted by Anandakumar *et al.* (2012) showed a decrease in RBC and Hb during the process of tumour formation. In addition, there was a decrease in WBC levels. This is because the benzo(α)pyrene compound is immunosuppressive. Although there was a decrease, WBC levels were still in the normal range. The results of the differential leukocytes at 5th weeks showed that there were still abnormalities in monocytes levels even though they had

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decreased from the previous week. Monocytosis still occurred at this time point presumably because the rats were under stress after the injection of benzo(α)pyrene solution to induce fibrosarcoma.

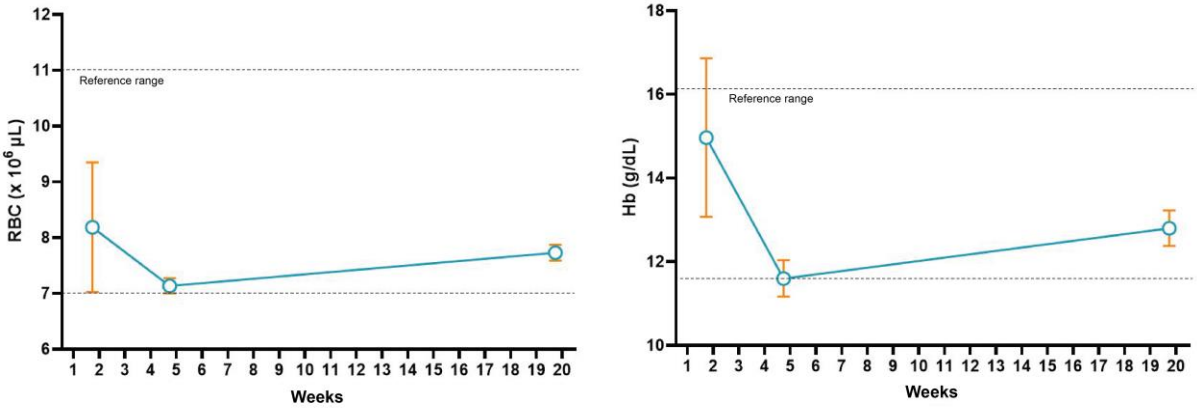


**Figure 1.** RBC and Hb levels from week 2 to week 20 (Graph depicted with mean ± SD)

In the 20th weeks, there was an upward trend in the RBC, PCV, WBC, and Hb. As previously described, cancer patients generally experience anemia, and sometimes have WBC levels below the normal range. Anemia in cancer patients is caused by malignant tumour invasion that causes haemorrhage. By its nature, cancer is invasive (Baba and Câtoi, 2007; Chauhan, 2010). In addition, cancer has massive vascularization directed at its cells which can cause fragmentation of erythrocyte cells (Chauhan, 2010). Haemorrhagic lesions are common because tumour vasculature tends to have increased permeability and proteolytic activity (Hodivala-Dilke *et al.*, 2003). However, in this study fibrosarcoma-bearing rats after treated with NDV did not experience anemia. Study of Komang *et al.* (2021) showed that rats with fibrosarcoma treated with NDV Tabanan-1/ARP/2017 isolate were able to relieve haemorrhagic lesions in the lungs. This is also related to the ability of the NDV Tabanan-1/ARP/2017 which can reduce tumour vascularization so that bleeding can be minimized (Sewoyo *et al.*, 2021b). WBC levels in the 20th weeks were slightly above the normal value. This implies that the administration of the NDV can trigger the immune system to help the oncolysis process. The triggered immune system implicated that NDV has been successfully replicated in tumour cells (Sewoyo *et al.*, 2021b). This is inversely proportional to cancer patients who generally have WBC levels below the normal range or leukocytopenia. In the process of growing larger mass, cancer cells form a strategy to escape the immune response. One way is to suppress the immune system. In the case of solid tumours in both animal and human models, there is a very high probability that individuals will experience immunosuppression caused by the release of transforming growth factor (TGF) -1 and TGF-β2 (Dun *et al.*, 2004; Hsieh *et al.*, 2000).

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For the differential leukocytes results in the 20th weeks, the abnormalities that occurred were neutrophilia, lymphopenia, and monocytosis. Lymphopenia or a decrease in lymphocyte levels is commonly observed in 20% of patients with advanced cancer in several types of cancer, one of which is sarcoma (Ménétrier-Cau *et al.*, 2019). In addition to cancer cells, solid tumours generally contain several types of cells, including stromal cells, endothelial cells, as well as several types of immune cells, including neutrophils.

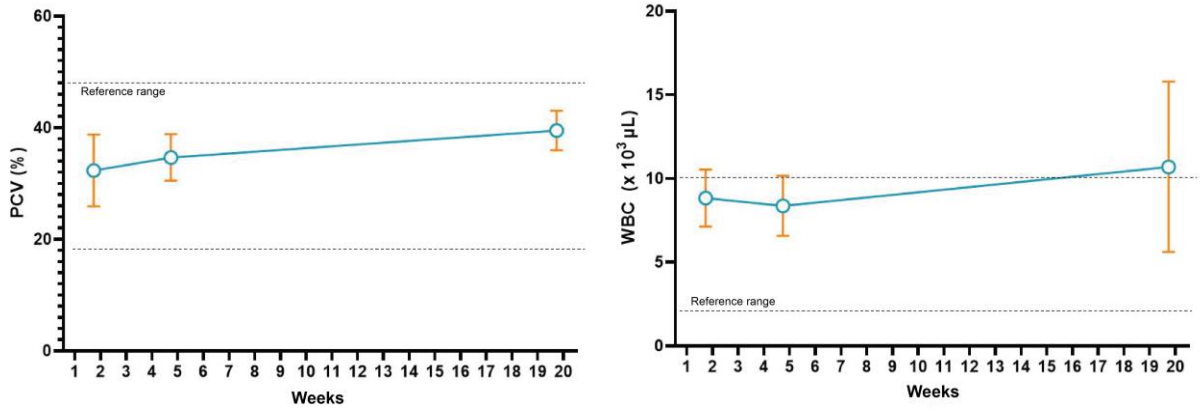


**Figure 2.** PCV and WBC levels from week 2 to week 20 (Graph depicted with mean ± SD)

Neutrophils can influence the tumour microenvironment, such as stromal, vascular, and other immune cells (Treffers *et al.*, 2016). Tumours that have a large size require a supply of oxygen and more nutrients, therefore the process of angiogenesis is needed to form new blood vessels. One of the angiogenesis processes can be promoted by matrix metalloproteinases-9 (MMP-9) (Treffers *et al.*, 2016). MMP-9 is produced predominantly by neutrophils (Deryugina and Quigley, 2015). The increase in neutrophil levels is in line with the increase in solid tumour size or tumour grade (Jensen *et al.*, 2009).

## Conclusion

Virotherapy with NDV has good potential because it does not cause adverse or pathological changes in the haematological profile. Rats treated with NDV had a haematological profile that tended to be better than cancer patients in general.

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## Suggestions

An obvious limitation of this study is the lack of control group. Further research is needed by using more replications and having a comparison/control group in order to get better and more accurate results. Furthermore, a high amount of replication also allows statistical testing of the significance between group. Research using NDV with different dose levels is also needed in order to observe its effect at different doses.

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