RESEARCH ARTICLE

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Role of *microRNA-486-5p* as Biomarker Response in Cell Line K562 of Chronic Myeloid Leukemia

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Abstract

Objective: This study aims to evaluate the efficiency and accuracy of microRNA (miR)-486-5p as a screening and diagnostic tool for chronic myeloid leukemia (CML). **Methods:** The study was performed on K562 cell line. The cell line of the erythroleukemia type originated from a 53-year-old female patient suffering from chronic myelogenous leukemia during a blast crisis. The cells are non-adherent, rounded in shape, and positive for the BCR: ABL1 fusion gene. In addition, we measured the expression of *miR-486-5p* in peripheral blood monocytes from healthy volunteers and compared the result with Imatinib treated and untreated K562 cell line. **Result:** As compared to control blood cells from healthy volunteers, there was a statistically significant downregulation of the expression of *miR-486-5p* in untreated K562 cells (p-value = 0.007). After Imatinib exposure, the *miR-486-5p* expression was significantly upregulated in K562 cells as compared to treated and untreated K562 cells (p-value = 0.004). **Conclusion:** Numerous reports demonstrate the role of miRNA in acting as oncogenes or tumor suppressors in various cancers. We have reported an alteration in the expression of *miR-486-5p* in the CML cell line. The upregulation of *miR-486-5p* expression in the post-imatinib exposure K562 cell line suggests that *miR-486-5p* has an onco-suppressor effector role in the BCR-ABL downstream signalling pathway. It is possible to investigate *miR-486-5p* as a potential biomarker for early CML detection.

Keywords: CML- BCR-ABL1- *miR-486-5p* expression- K562 cell Line

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Introduction

A genetic abnormality in the Philadelphia chromosome causes chronic myeloid leukemia (CML) by forming the fusion gene *BCR-ABL* [1]. This gene produces an aberrant protein that contributes to the proliferation and survival of myeloid cells. Myeloid cells, a type of white blood cell that often aids the body in battling infections, develop out of control, which is one of its defining characteristics [2, 3]. CML has an annual incidence of 1-2 per 100.000, and it makes up 7–20% of adult leukemia. Clinically, it is characterized by an overabundance of myeloid cells that develop and function appropriately [4]. The most prevalent kind of adult leukemia in India is CML [5].

CML has been a model disease in modern haematology/oncology, from the recognition of a cytogenetic abnormality to bone marrow transplantation, PCR-guided treatment, and the introduction of tyrosine kinase inhibitors [6, 7]. The expression profile of cancer miRNAs can predict the prognosis and clinical response to treatment [8]. Several groups have discovered a class of regulatory genes, the miRNAs, that are mixes pressed in CML, paving the way for CML biomarker research [9]. A Philadelphia chromosome, which causes the *BCR-ABL* fusion protein to develop, is a hallmark of CML, a hematological malignancy [8]. Tyrosine kinase inhibitors (TKIs), such as Imatinib, revolutionized the treatment of CML and significantly improved patient outcomes. Nevertheless, not all CML patients respond to TKI therapy similarly, and some exhibit resistance or relapse [10, 11]. Researchers have investigated the role of *miR-486-5p* in the setting of many malignancies, including leukemia. It has been shown to play a role in controlling cell proliferation, differentiation, and apoptosis, and is one of the most prevalent miRNAs in hematological tissues [12, 13]. For instance, studies using the K562 cell line, a popular model for CML research [14], have examined the significance of *miR-486-5p* in predicting therapy response in CML.

Anuupama Suchiita et al. [14] published a study in 2021 that looked at the link between *miR-486-5p* expression and how well TKIs worked as a treatment for CML patients. The study discovered that CML patients with higher levels of *miR-486-5p* expression responded better to TKI therapy. They had higher rates of both complete cytogenetic response and major molecular response. The researchers also showed that *miR-486-5p*

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directly targeted the *BCR-ABL* fusion gene, stopping it from being expressed and making CML cells more sensitive to TKIs [14]. While these studies suggest that *miR-486-5p* may have predictive value in determining treatment response in CML, it's important to note that research in this field is still ongoing. Further studies are needed to validate these findings in larger patient cohorts and explore the underlying molecular mechanisms involved. Additionally, the application of *miR-486-5p* as a predictive biomarker in clinical settings would require comprehensive clinical trials and validation studies. miR-486- 5p has been shown to play a role in regulating the growth and differentiation of myeloid cells.

This aim of study was to estimate the *BCR-ABL*1 and *microRNA 486-5p* expression in a CML K562 cell line before and after exposure to an Imatinib mesylate, a tyrosine kinase inhibitor.

Materials and Methods

The department of biochemistry at Indira Gandhi Institutes of Medical Sciences, Patna, India, collaborated with the departments of oncology (State Cancer Institute) and pathology to carry out this study. We obtained the CML K562 Cell Line from the National Centre for Cell Science in Pune, India. K562 cell line cells are of the erythroleukemia type, and this line was derived from a 53-year-old female chronic myelogenous leukemia patient in a blast crisis. The cells are non-adherent, rounded in shape, and positive for the BCR: ABL1 fusion gene. All the media and chemicals used are of high purity. The study was approved by the Institute's Ethics Committees.

Maintaining of the K562 CML Cell Line

It had 10% fatal bovine serum (FBS), 100 U/ mL penicillin, 100 g/mL streptomycin, 250 ng/mL amphotericin, 250 g/mL gentamycin, and 250 ng/mL of each. This is what we used to grow K562 cells. We maintained the cultures at 37 °C, in a 5% CO_2 atmosphere, and under normal humidified conditions.

IC_{50} of Imatinib in K562 Cells

We performed Trypan blue exclusion testing to determine cell viability. The 50% proliferation inhibitory concentration (IC_{50}) of Imatinib was investigated in the *BCR-ABL1*+ve CML cell line. Imatinib mesylate (Sigma, USA) was dissolved in sterile distilled water as a 20 mM stock solution and stored at 4oC in the dark. We prepared working dilutions in 2% FBS containing RPMI medium and passed them through a 0.22 M filter. After incubation for three days when cells were 80–90% confluent, the cell culture plate was centrifuged at 2000 rpm for 10 minutes, and the supernatant media with 10% FBS was discarded. We washed the cells with 100L of phosphate buffered saline (PBS). To treat the cells, 100µL of RPMI with 2% FBS and a concentration of Imatinib ranging from 10µM to 100µM was added to each well until there was a total of 100µL in each well. We incubated the cell culture plate at 37oC for 48 hours in a CO2 incubator. After incubation, using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] test, an assay was performed. MTT is a colorimetric assay that measures cytostatic activity, or cell proliferation rate. The percentage of inhibition for each Imatinib concentration was calculated as: [(Absorbance of blank - Absorbance of treated cells) / (Absorbance of blank)] × 100. In this study, the growth of K562 cells was inhibited by 50% at a 4 µM concentration of Imatinib.

Exposure of K562 Cells to Imatinib

We prepared two sets of K562 cells from the same passage number, each consisting of 3 T-25 flasks containing 500,000 cells in 5 mL of complete RPMI media with 10% FBS. The flasks were placed in a CO_2 incubator, and cells were allowed to grow for 3 days, till they reached confluence. After centrifugation, the media was removed, and cells were washed with PBS. In a T-25 flask containing RPMI 1640 media with 2% FBS and antibiotics, we seeded washed cells from each flask once more. In set 1 (3 flasks), Imatinib at 4 µM concentration was added, and set 2 (3 flasks) was without Imatinib. We kept both sets 1 and 2 in a CO_2 incubator for 48 hours. The aliquots of cells from each flask were processed for RNA extraction; hence, there were a total 25 samples of Imatinib-treated K562 cells and 25 samples of K562 cells without Imatinib treatment.

Purification of Peripheral Blood Monocytes from Healthy Volunteers

Using a Ficoll-Paque density gradient centrifuge, we separated peripheral blood mononuclear cells (PBMCs) from whole blood samples of healthy participants. We took monocytes from PBMCs because they liked sticking to plastic. We then put them in three T-25 flasks with full concentrations of RPMI 1640 growth medium (set 3) and used them as healthy control cells.

Estimation of miR-486-5p Expression in Control Peripheral Blood Monocytes and in K562 Cells (Before and After Imatinib Exposure)

After the cells were incubated in CO2 for 48 hours in each of the three sets of T-25 flasks, we removed aliquots of the cells (in triplicate) for RNA extraction and additional processing.

Total RNA Extraction

Following the manufacturer's instructions, we extracted total RNA (including miRNAs) from K562 cells (imatinib-treated and untreated) and healthy peripheral blood monocytes using the modified acid guanidinium thiocyanate, phenol, and chloroform (AGPC) method [15] and the Trizol reagent (Invitrogen Life Technologies).

Synthesis of Complementary DNA (cDNA)

We performed an RNA integrity check, polyadenylations the microRNAs with poly(A) polymerase and ATP, and then created cDNA from poly(A) tailed microRNAs using the Affinity Script qPCR First-strand cDNA synthesis kit (Agilent Technologies, USA). The oligo-dT adapter primer included a special sequence at its 5′ end that made it possible to amplify cDNAs using qRT-PCR.

MiR-486-5p quantification via qRT-PCR

We performed qRT-PCR in a Rotor-Gene Q real-time PCR cycler from Qiagen using SYBR Green qPCR Master mix (Thermos Fisher Scientific, USA), a *miR-486-5p*-specific forward primer (UCCUGUACUGAGCUGCCCCGAG), and a universal reverse primer (annealing temperature 58 °C). To guarantee the PCR product's specificity, a melting curve covering a 35–95 °C temperature range was created and presented on Figure 1(a). We detected and presented the Ct values for *miR-486-5p* on Figure 1(b), using the internal control RNA gene (RNU6B) as a baseline. The matching $2^{-\Delta Ct}$ values demonstrate the expression of *miR-486-5p* in K562 cells and its control over peripheral blood monocytes.

Statistical Analysis

Statistical analyses were performed using Graph SPSS software version 29 and Microsoft Excel. We compared the quantitative and qualitative data separately. We defined a p-value of less than 0.05 as statistically significant.

Results

Experimental study in K562 cell line

For the experiments, there are three sets of cells, each with 23 samples. Set 1 had K562 cells that had not been treated, Set 2 had K562 cells that had been treated with 4µM imatinib (10 times less than standardized 1050), and Set 3 had control monocytes from healthy volunteers that had not been treated with any drugs. We extracted total RNA from the above group of cells and analysed it for *BCR-ABLL* and *miR-486-5p*.

BCR-ABL1/ABL1 ratio (NCN) in K562 cell line

We measured the *BCR-ABL* 1/ABL1 ratio (NCN) in untreated and imatinib-treated K562 cells. Figure 2 presents a comparison of these two levels. Table 1 presents a statistically significant downregulation of the *BCR-ABL1* fusion gene in imatinib-treated K562 cells, with a p-value of less than 0.001.

Table 1. BCR-ABL1/ ABLI Ratio (NCN) in Untreated and Imatinib Treated CML K562 Cell Line

BCR-ABL1/ABL1 ratio (NCN)	Mean ± SD	p-value
Untreated CML K562 cell line	125.37 ± 9.34	< 0.001*
Imatinib treated CML K562 cell line	71.06 ± 14.93	

*p-value calculated by Mann - Whitney U Test

Table 2. $2^{-\Delta Ct}$ The Expression of *miR-486-5p* in K562 Cells Compared with Untreated K562 Cells and Control Normal Blood Monocytes

miR-486-5p ($2^{-\Delta Ct}$)	Mean±SD	Median	P value
In control monocytes	0.26 ± 0.195	0.18	0.007
In CML K562 cell line (untreated)	0.05 ± 0.047	0.04	

Expression of MIR-486-5P in K562 cell line and control monocytes

We used qRT-PCR to look at the expression of *miR-486-5p* in healthy volunteer control monocytes and in the CML K562 cell line (untreated and imatinib-treated), as presented on Figure 3. This study found that untreated K562 cell line cells had lower *miR-486-5p* expression than control monocytes at present (Table 2). We discovered that untreated K562 cell line cells significantly downregulated *miR-486-5p* more than control monocytes (p-value 0.007). After that, we found Imatinib-treated K562 cell line cells showed higher expression of *miR-486-5p* compared to untreated K562 cell line cells (Table 3). This indicates a significant upregulation of *miR-486-5p* expression in K562 cell line cells following Imatinib exposure, compared to both treated and untreated K562 cells (p-value = 0.004).

Discussion

Small non-coding RNAs called microRNAs, which

Table 3. $2^{-\Delta Ct}$ The Expression of *miR-486-5p* in K562 Cells Compared with Imatinib-treated and Untreated K562 Cells

miR-486-5p ($2^{-\Delta Ct}$)	Mean±SD	Median	P value
CML K562 cell line (treated with Imatinib)	0.15 ± 0.136	0.1	
In CML K562 cell line (untreated)	0.05 ± 0.047	0.04	0.004

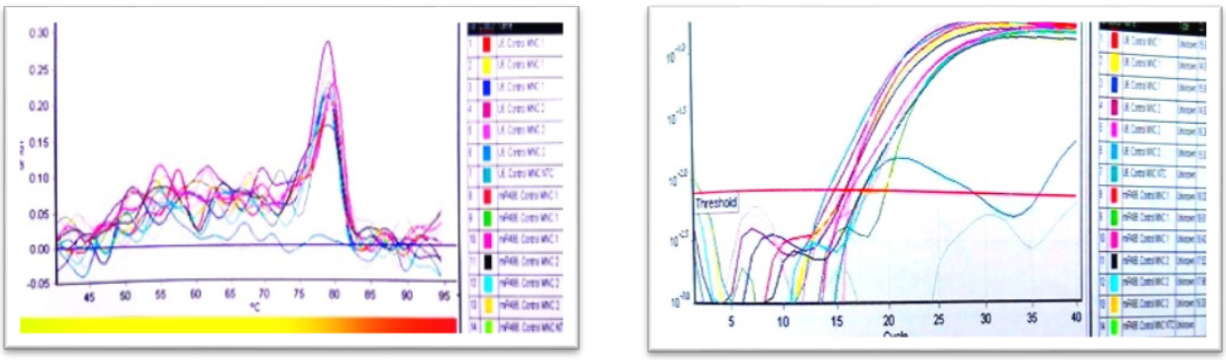

Figure 1. (a), Melting curve analysis for miR-486-5p in CML K562 cell line. Real time amplification graph for miR-486-5p with internal control RNU6B in CML K562 cell line

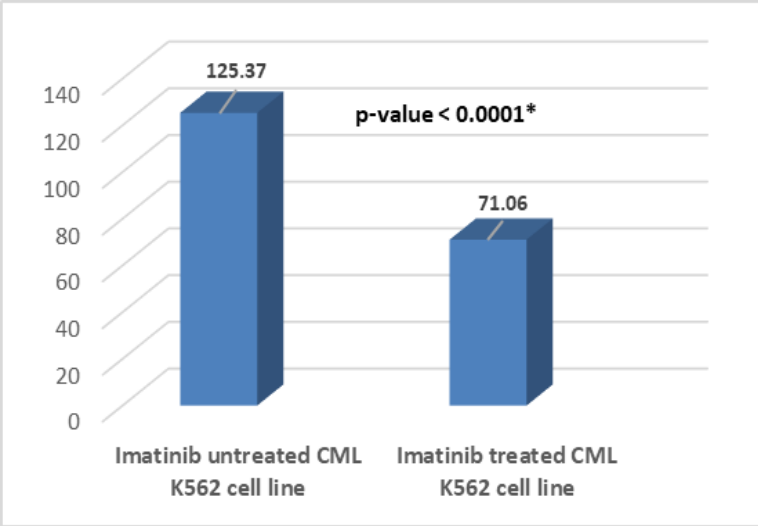

Figure 2. BCR-ABLI/ ABLI Ratio (NCN) in Untreated and Imatinib Treated CML K-562 Cell Line

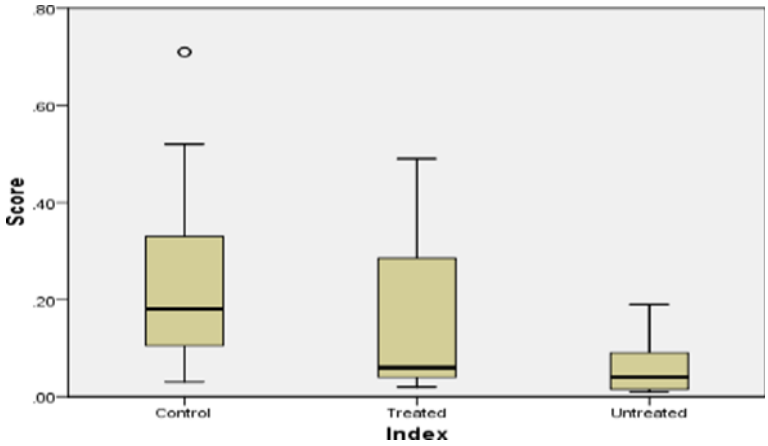

Figure 3. Expression of *miR-486-5p* in K562 Cells Treated with Imatinib, Untreated K562 Cells, and Control Normal Blood Monocytes

range in length from 20 to 24 nucleotides, play a key role in post-transcriptional gene silencing and act as significant regulators of gene expression. They control a lot of oncogenes and tumor suppressor genes to change how they are expressed. This changes how important biological processes like cell division, proliferation, and death are expressed. Microarray-based expression profiling has demonstrated that dysregulated microRNA expression in many malignancies is the norm rather than the exception. Researchers have assigned conflicting roles to the *miR-486-5p* in various solid tumors, including those of a tumor suppressor and a tumor promoter. Numerous studies have demonstrated the downregulation of *MiR-486-5p* in non-small cell lung cancer, demonstrating an inverse correlation between its expression and tumor growth, invasion, and metastasis [16-20]. Wang et al. [18] demonstrated that *miR-486-5p* targets the pro-tumorigenic gene ARHGAP5 in non-small cell lung cancer. According to Oh et al. [21], *miR-486-5p* acts as a tumor suppressor in gastric cancer and explains its anti-oncogenic effect by targeting and blocking the anti-apoptotic OLFM4 gene. In their study of colorectal cancer tissues, Liu et al. [22] found a comparable decrease in the expression of *miR-486-5p* and established the neuropilin-2 gene as its specific direct functional target. Yi et al. observed a reduction in *miR-486-5p* expression in esophageal squamous cell cancer [23]. They established that *miR-486-5p*'s detrimental impact on cellular migration is what causes it to have an anti-oncogenic effect. Zhang et al. [24] reported a dramatic reduction in *miR-486-5p* expression in metastatic prostate cancer tissues compared to localized prostate cancer. This group also confirmed that Snail is a *miR-486-5p* target gene. Snail is a key regulator of the epithelial-to-mesenchymal transition. Rask et al. [25] found that *miR-486-5p* expression was significantly lower in lymph node-positive breast invasive ductal carcinoma tissues than in lymph node-negative breast carcinomatous tissues. In their study of breast cancer, Zhang et al. [26] discovered that increasing the amount of *miR-486-5p*, which targets the PIM-1 oncogene, greatly slowed down cell growth both in the lab and in living things, and boosted cell death. Despite a large number of studies supporting its involvement, only a few papers have revealed the upregulation of *miR-486-5p* and its role as an oncogene

in solid tumors. Goto et al. [27] found that the increased expression of miR-486 in tumors was associated with a lower cancer-specific mortality in patients with stage III and IV renal cell carcinoma. In aggressive and metastatic pancreatic ductal carcinomas, Rajesh Kumar et al. [28] discovered that the *miR-486-5p* was overexpressed.

Only one prior study has examined the relevance of *miR-486-5p* in hematological malignancies. When Wang et al. [29] looked at CD34+ stem cells from CML patients' bone marrow, they found that they had higher levels of *miR-486-5p* expression compared to normal peripheral blood stem cells. This was especially true for the megakaryocyte-erythroid progenitor population. They also showed that increasing *miR-486-5p* led to better growth and survival of CML progenitor cells, and this was linked to both *BCR-ABL* kinase-dependent and kinase-independent processes. This team demonstrated that *miR-486-5p*'s growth-promoting effects in CML progenitor cells stemmed from its targeting of the *FOXO1* and *PTEN* genes, under the control of *PI3K/AKT* signalling. In the current study, CP-CML patients' peripheral blood lymphocytes had lower levels of *miR-486-5p* than those of healthy volunteers. The results back up the idea that *miR-486-5p* works as an oncosuppressor in CML and are similar to what was found in the *BCR-ABL*1+ve K562 cell line. We believe that this is the first description of the *miR-486-5p* expression pattern in peripheral blood leukocytes from CML patients. Wang et al. [29] showed that *miR-486-5p* levels went up in CD34+ stem/progenitor cells in the bone marrow of people with CML, which is different from what we found. We have noticed changes in the expression of miRNAs during the development of specific hematopoietic stem cell lineages [30]. According to Goto et al. [27], *miR-486-5p* expression may be a potential prognostic factor in cancer. Patients in the current study who had higher levels of *miR-486-5p* expression had better prognoses and responded to imatinib in a shorter amount of time, and vice versa, according to a link between pre-treatment *miR-486-5p* levels, Sokal score, and THR. We agree with Yang et al. [31], who discovered that giving propofol to lung cancer cell lines (H1299 and H1792 cells) increased levels of miR-486, which led to more cell death. However, treatment with the miR-486 antagonist reversed this effect.

Action of miR-486-5p in CML

One of the crucial signalling pathways governed by *BCR-ABL* activity in CML is the PI3K pathway [32]. P110 and P85 are the two subunits that make up *PI3K*. *PIK3R1* codes for the protein p85, which acts as an adapter and connects the p110 catalytic unit to the plasma membrane by attaching to activated protein-tyr kinases, such as BCR/ABL. Activated p110 phosphorylates PIP2 to PIP3, which then binds to the domain of the cancer-associated serine/threonine kinase AKT [15]. The *PIK3R1*-encoded protein p85α is a key regulator in the activation of the PI3K-AKT pathway in a number of cancers, including CML. Huang et al. [33] say that *miR-486-5p* lowered the levels of p85 in hepatocellular carcinoma at both the mRNA and protein levels by connecting to the 3′-UTR region of *PIK3R1*. Overexpression of *miR-486-5p* dramatically decreased in vitro migration and invasion of hepatocellular carcinoma cells, while it prevented in vivo cell growth. MiR-486-5p directly targets the expression of *PIK3R1*, thereby inhibiting the activation of the *PI3KAKT* pathway.

The *BCR-ABL1* oncogene downregulates *miR-486-5p*, and overexpresses miRNAs like *PIK3R1*, leading to oncogenesis. Our investigation's clearly shows higher expression of *miR-486-5p* in the post-imatinib CML samples. Based on these results, we think that *BCR-ABL1, miR-486-5p, PIK3R1, PIP3,* and *AKT* are the ways that CML changes into a cancer. In conclusion, our study found that the molecular mechanisms behind the deregulation of *miR-486-5p* in CML and the identification of its target genes require further study.

In conclusion, numerous reports demonstrate the role of miRNA in acting as oncogenes or tumor suppressors in various cancers. This present study has reported an alteration in the expression of *miR-486-5p* in the CML cell line. We have associated the distinct increase in *miR-486-5p* expression in the CML+ve K562 cell line in response to Imatinib with the TKI activity of the *BCR-ABL*1 fusion gene, which its downstream effector pathways inhibit. This mechanism's reliability requires more research. We think this is the first report of a change in *miR-486-5p* expression in peripheral blood leukocytes from the CML+ve K562 cell line. Despite the ongoing challenges, the potential advantages of employing *miR-486-5p* as a biomarker are substantial.

Author Contribution Statement

The responsibilities of the First Author/Corresponding Author include conceptualizing the funding acquisition, developing the methodology, conducting formal analysis, validating the findings, and writing the original draft. The Co-Author is responsible for the writing process, including review and editing, resource management, validation, data curation, and visualization. The article received endorsements from all authors.

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General

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Scientific Approval

The study was part of a PhD thesis approved by the Research Committees of Indira Gandhi Institute of

Medical Sciences, Patna, in 800014.

Ethical Declaration

The Ethics Committees of the Indira Gandhi Institute of Medical Sciences in Patna approved the study.

Data Availability

This published article and its Additional Files contain all of the data created or analysed throughout this investigation.

Study Registration

This study was not registered in any registering dataset (for clinical trials, guidelines, or meta-analyses). Because it is not applicable.

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