e-ISSN: 3023-655X

# Ankyra Medical Journal

Volume: 5 Issue: 2 Year: 2026


MHA
MEDIHEALTH ACADEMY

## EDITOR-IN-CHIEF



## ASSOCIATE EDITORS-IN-CHIEF



## EDITORIAL BOARD

Ankyra
Medical Journal

# Contents

Volume: 5 Issue: 2 Year: 2026

## ORIGINAL ARTICLES



## REVIEW



## CASE REPORT



## LETTER TO THE EDITOR

Ankyra Medical Journal
Original Article
DOI: 10.51271/ANKMJ-0063

# The effects of visual information leaflets on perioperative anxiety in pediatric patients

Zehra Betül Güzel[1], Esra Polat[2], Gülçin Aydın*[3], Işın Gençay[3], Ünase Büyükkoçak[4]

[1]Department of Anesthesiology and Reanimation, Pursaklar State Hospital, Ankara, Turkiye
[2]Department of Anesthesiology and Reanimation, Kastamonu Training and Research Hospital, Kastamonu, Turkiye
[3]Department of Anesthesiology and Reanimation, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkiye
[4]Department of Anesthesiology and Reanimation, Lösante Children's and Adult Hospital, Ankara, Turkiye

**Cite this article:** Güzel ZB, Polat E, Aydın G, Gençay I, Büyükkoçak Ü. The effects of visual information leaflets on perioperative anxiety in pediatric patients. *Ank Med J.* 2026;5(2):33-39. doi:10.51271/ANKMJ-0063

**Received**: 07/12/2025 ◆ **Accepted**: 06/01/2026 ◆ **Published**: 25/03/2026

## ABSTRACT

**Aims:** The aim of this study was to investigate the beneficial effects of a visual information leaflet designed in our clinic on perioperative anxiety in pediatric patients scheduled for general anesthesia, as well as on anxiety levels of their caregivers.

**Methods:** One hundred pediatric patients American Society of Anesthesiologists (ASA) risk classification I–II, aged 4 to 12 years, were randomly divided into two groups. During the preoperative visit, the control group (n=47) received only the standard written information form routinely used in our clinic, whereas the study group (visual group; n=53) received an additional visual information leaflet alongside the standard written form. Anxiety and pain levels of the children were assessed using validated scales before and after surgery. Negative behaviors in children were also evaluated during the postoperative period. In addition, anxiety levels of patient attendants were assessed using appropriate scales.

**Results:** Preoperative anxiety levels in children and the incidence of negative behaviors in the postoperative period were significantly lower in the visual group. However, in both groups, preoperative anxiety levels and the incidence of delirium increased as the age of the children decreased. Preoperative concerns of patient attendants were significantly reduced in the Visual group, and these concerns were also lower in the postoperative period. Furthermore, baseline anxiety levels, both preoperatively and postoperatively, were lower among attendants who received the visual information leaflet. No statistically significant difference was observed between the groups with respect to postoperative pain scores.

**Conclusion:** The use of a visual information leaflet describing the anesthesia procedure with pictures, in addition to the standard written information form, appears to reduce anxiety and concerns related to anesthesia in both pediatric patients and their attendants. This approach may also reduce postoperative complications and negative behavioral outcomes in children.

**Keywords:** Visual information leaflet, pediatric anesthesia, premedication

## INTRODUCTION

Anxiety prior to medical treatment is common and often unavoidable in pediatric patients, posing significant challenges for both children and their families.[1] High perioperative anxiety leads to numerous adverse outcomes, including prolonged anesthesia induction time, increased incidence of postoperative delirium, negative postoperative behavioral changes, increased postoperative pain and analgesic consumption, agitation, prolonged hospital stay, delayed wound healing, deterioration of vital signs, and impaired postoperative compliance.[2]

Studies have shown that preoperative parental anxiety has significant negative effects on children in terms of anxiety levels and emotional responses. The anxiety levels of family members are closely associated with children's postoperative recovery.[3,4]

Various approaches to alleviate preoperative anxiety have been widely studied, including preanesthetic medications, distraction techniques, parental presence during anesthesia induction, and preoperative psychological and educational interventions. Among these, non-pharmacological management of anxiety offers important advantages over anxiolytic medications, as it is not associated with adverse effects and emphasizes the importance of psychological assessment and preparation in the preoperative period.[5]

Emotions such as fear and anxiety often arise from a lack of communication and insufficient information. Accordingly,

*Corresponding Author: Gülçin Aydın, drgulcinaydin@yahoo.com

various perioperative psychological preparation methods have become increasingly widespread in recent years. Reviews of the literature indicate that different visual interactive methods applied to patients are more effective in reducing preoperative anxiety levels compared with control groups.[6]

In this study, we aimed to investigate the potential benefits of providing a visual information leaflet in addition to the standard written information form on perioperative anxiety levels in pediatric patients scheduled for general anesthesia, as well as in their patient attendants.

## METHODS

The study was carried out with the permission of the Kırıkkale University Faculty of Medicine Hospital Scientific Researches Evaluation and Ethics Committee (Date: 19.04.2016, Decision No: 11/02). We obtained an informed consent form from all patients for procedure. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study included children aged 4 to 12 years who were scheduled for elective surgery, as well as their patient attendants. Pediatric patients classified as American Society of Anesthesiologists (ASA) physical status I–II and without cognitive, hearing, or speech impairments that could interfere with communication were eligible for inclusion. Patient attendants who had no cognitive, hearing, or speech impairments, no known psychological disorders, were literate, and willing to participate in the study were also included.

Children younger than 4 years or older than 12 years, those classified as ASA III or higher, children undergoing emergency surgery, children with difficult intubation, and those who developed intraoperative surgical and/or anesthesia-related complications (such as respiratory depression, myocardial depression, cardiac arrhythmias, bronchospasm, laryngospasm, anaphylactic reactions, hypotension, or bleeding) were excluded from the study. In addition, illiterate patient attendants, attendants with communication or psychological problems, and those who declined to participate were excluded.

Using a closed-envelope randomization method, pediatric patients and their attendants were randomly assigned to one of two groups during their preoperative evaluation at the anesthesia outpatient clinic:

**Control group:** Information provided only through the standard written information form (n=47)

**Visual group:** Information provided through the standard written information form in addition to a visual information leaflet describing the anesthesia procedure with pictures designed in our clinic (n=53) (**Figure**).

The age and sex of the patients and the department in which the surgery was performed (pediatric surgery, dentistry, ophthalmology, otorhinolaryngology, orthopedics, and urology) were recorded. The age, sex, and educational level of the patient attendants were also documented.

The study was conducted in three phases: the anesthesia outpatient clinic, the preoperative bedside, and the postoperative bedside, as described below.

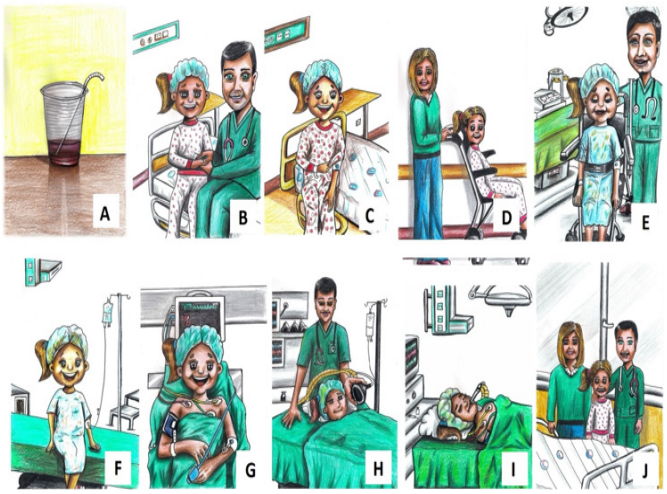


**Figure 1.** Visual materials used for anesthesia information
(A) A glass of cherry juice and midazolam mix is to drink 30 minutes before the operation. It is explained that this mix is to be drunk before the operation.
(B) Establishing vascular access at the bedside.
(C) Detection of the vascular access. The patient is informed that they may feel a slight pain when establishing vascular access, and that it will be covered by the medical tape.
(D) Transfer from the service to the operating room. The patient is told that he/she will be taken to the preoperative preparation room with his/her parents by wheelchair.
(E) Wearing surgical clothes. In the preparation room, the patient is told that children should put on the clothes that they will wear in the operating room. He/she is told that his/her parents will not be able to accompany him/her after this stage.
(F) Entering the operating room and preoperative preparation-1. The patient is told in an appropriate way that he/she will be taken into the operating room where he/she will lie on the green-colored bed, as seen in this picture, and that the machines around him her will help him/her to sleep.
(G) Entering the operating room and preoperative preparation-2. After the patient is taken to the operating room, he/she is told that some tapes (electrodes) will be applied, and that there will be a blue peg (saturation probe) on his/her thumb, which will not hurt. He/she will be told that he/she will wear a device that will take measurements by applying pressure at intervals to his/her arm, and that the water that he/she did not drink at night will be replaced by fluids through the vascular access. The patient is informed that none of the operations the patient will undergo will hurt.

### Anesthesia Outpatient Clinic

Children in the control group and their attendants were informed using the standard written information form routinely used in the clinic. In addition to this standard form, pediatric patients and patient attendants in the visual group were provided with a visual information leaflet illustrating each step of general anesthesia using 10 caricature-style images. This leaflet was designed in our clinic and drawn by a professional technical draftsman. The illustrations depicted situations that patients would encounter in the patient bed, premedication room, and operating room using cartoon-style images. Written permission was obtained from the artist for the use of these illustrations.

Baseline anxiety levels of patient attendants in both groups were assessed using the State–Trait Anxiety Inventory (STAI) (**Table 1**), one of the most widely used self-report measures of anxiety. Developed by Spielberger et al., this inventory assesses how individuals feel under specific conditions and consists of 20 items: 10 items reflecting negative emotions expressed directly and 10 items reflecting positive emotions expressed in reverse. Higher scores indicate higher anxiety levels.[7,8]

In addition, a questionnaire consisting of 11 items, each scored from 1 to 5, was used to assess anxiety regarding anesthesia (AIE). Higher scores indicate increased AIE.[9]

Both the STAI and AIE scales were re-administered to patient attendants in the postoperative period.

**Table 1.** State Anxiety Inventory (STAI-S) items

| Item | Statement | Never (1) | Sometimes (2) | Often (3) | Always (4) |
|---|---|---|---|---|---|
| 1 | I feel calm right now | 1 | 2 | 3 | 4 |
| 2 | I feel secure | 1 | 2 | 3 | 4 |
| 3 | I feel tense | 1 | 2 | 3 | 4 |
| 4 | I feel regretful | 1 | 2 | 3 | 4 |
| 5 | I feel at ease | 1 | 2 | 3 | 4 |
| 6 | I feel unpleasant | 1 | 2 | 3 | 4 |
| 7 | I am worried about possible misfortunes | 1 | 2 | 3 | 4 |
| 8 | I feel rested | 1 | 2 | 3 | 4 |
| 9 | I feel anxious | 1 | 2 | 3 | 4 |
| 10 | I feel relaxed | 1 | 2 | 3 | 4 |
| 11 | I feel self-confident | 1 | 2 | 3 | 4 |
| 12 | I feel upset | 1 | 2 | 3 | 4 |
| 13 | I feel very nervous | 1 | 2 | 3 | 4 |
| 14 | I feel extremely tense | 1 | 2 | 3 | 4 |
| 15 | I feel relaxed | 1 | 2 | 3 | 4 |
| 16 | I feel satisfied | 1 | 2 | 3 | 4 |
| 17 | I feel confused due to excitement | 1 | 2 | 3 | 4 |
| 18 | I feel joyful | 1 | 2 | 3 | 4 |
| 19 | I feel happy | 1 | 2 | 3 | 4 |
| 20 | I feel cheerful | 1 | 2 | 3 | 4 |

### Preoperative Patient Bedside

On the morning of surgery, while patient attendants were present, children's anxiety levels were assessed at the bedside using the Modified Yale Preoperative Anxiety Scale (mYPAS) (Table 2) prior to premedication with midazolam (Dormicum®, Deva Holding A.Ş., Turkiye). The mYPAS is an observational tool used to evaluate preoperative anxiety in children aged two years and older. The scale consists of five domains: activity, vocalization, emotional expressivity, state of apparent arousal, and use of parents.[10,11]

### Postoperative Patient Bedside

In the recovery room, the pediatric anesthesia emergence delirium (PAED) (Table 3) scale was used to assess early postoperative agitation. This scale incorporates cognitive-based assessments in addition to agitation-related behaviors. A PAED score greater than 10 is considered sensitive and specific for emergence agitation, with higher scores indicating more severe agitation.[12]

Postoperative pain was evaluated using the Facial Expression Scale (FES), also known as the Wong–Baker Pain Scale, at 0, 1, 2, and 6 hours postoperatively, and postoperative analgesic requirements were recorded. This scale uses six facial expressions to rate pain intensity from 0 to 10, ranging from a smiling face indicating no pain to a crying face indicating severe pain.[13]

Patient attendants were contacted by telephone on postoperative days 2 and 14 after hospital discharge and were asked about any behavioral changes in their children, including eating disorders, sleep disturbances, urinary incontinence, and other behavioral alterations that may occur in the early postoperative period. For patients who had not yet been discharged, these questions were asked at the bedside.

### Statistical Analysis

At the end of the study, the collected variables were found not to be normally distributed or homogeneously distributed between the groups. Therefore, the Mann–Whitney U test was used to compare variables between the two groups, with a p-value of <0.05 considered statistically significant. The Wilcoxon signed-rank test was used to compare paired measurements within each group (STAI and AIE scores), with a p-value of <.05 considered statistically significant. Correlations between variables were analyzed using Spearman's correlation test, with p-values of <0.05 considered statistically significant.

## RESULTS

The study was conducted with a total of 100 patients and their patient attendants. The visual group contained 24 female and 29 male patients, with a median age of seven, while the control group contained 20 female and 27 male patients, with a median age of five. No differences were observed between the groups in the age, sex, type of operation, number of previous operations, number of children in the family and number of child in the family variables. The degree of relation, age and educational status of the patient attendants were found to be similar in both groups (Table 4).

When the test results were examined, the preoperative AIE ($Z=-3.605$, $p<0.001$) and STAI ($Z=-0.839$, $p=0.005$) scores and the postoperative AIE ($Z=-2.149$, $p=0.032$) scores were found to be different, and both the preoperative and postoperative AIE scores in the visual group were observed to be lower. Preoperative STAI scores were also found to be lower in the visual group, while the MYPAS scores were observed to be different in both groups, although the scores in this test were lower in the Visual group regarding numerical values.

**Table 2.** Modified Yale Preoperative Anxiety Scale (mYPAS)

| Score | Description |
|---|---|
| **Activity** | |
| 1 | Interested in surroundings, curious, playing with toys, active movement within the room |
| 2 | Uninterested in surroundings, not playing, fidgety hands, sucking fingers, sitting close to parent |
| 3 | Moving toward toys without focus, excited, restless on chair, pushing mask away, clinging to parent |
| 4 | Actively trying to escape, pushing with arms and legs, whole-body movement, running around the room, not interested in toys, desperately clinging to parent |
| **Vocalization** | |
| 1 | Responds normally to adults |
| 2 | Responds to adults only with baby talk or head nodding |
| 3 | Quiet and does not respond to adults |
| 4 | Whimpering, moaning, softly crying |
| 5 | Crying and shouting "no" |
| 6 | Crying loudly and continuously, screaming audibly even under the mask |
| **State of apparent arousal** | |
| 1 | Alert, occasionally scanning the environment, watching or noticing the clinician's actions |
| 2 | Withdrawn, sitting quietly and motionless, sucking finger, turning face toward parent |
| 3 | Vigilant, rapidly scanning surroundings, startled by environmental sounds, frightened eyes, tense |
| 4 | Anicked, crying, pushing others away, attempting to leave the area |
| **Emotional expressivity** | |
| 1 | Clearly happy, smiling, or focused on play |
| 2 | Neutral facial expression, no clear emotional emphasis |
| 3 | Fearful, anxious, sad, or tearful |
| 4 | Distressed, crying, frightened with wide eyes |
| **Use of parent** | |
| 1 | Playing independently, sitting quietly, no need for parent; engages if parent initiates interaction |
| 2 | Interacting with parent, leaning toward parent, quietly talking, resting against parent |
| 3 | Quietly watching parent, observing movements, avoids eye contact; either accepts suggestions or clings to parent |
| 4 | Keeps parent at a distance or avoids parent; may push parent away or cling desperately and refuse separation |

The postoperative complaint levels (Z =-3.613, p<0.001) were found to be different in both groups, and the complaint rate was lower in the visual group (Table 5, 6). In a comparison of

**Table 4.** Descriptive table of the demographic data of the study groups

| Group | Variable | Min | Max | Median | SD |
|---|---|---|---|---|---|
| Control | Age | 4 | 12 | 5 | 2.68 |
| | Sex | 1 | 2 | 2 | 0.50 |
| | Siblings | 1 | 6 | 2 | 1.05 |
| | Which child | 1 | 5 | 2 | 1.08 |
| | Complaint | 0 | 1 | 1 | 0.47 |
| | Patient attendant | 1 | 2 | 1 | 0.28 |
| | Patient attendant's age | 21 | 50 | 34 | 6.06 |
| | Educational status of the patient attendants | 0 | 4 | 3 | 1.10 |
| Visual | Age | 4 | 12 | 7 | 2.52 |
| | Sex | 1 | 2 | 2 | 0.50 |
| | Siblings | 1 | 6 | 2 | 0.96 |
| | Which child | 1 | 6 | 2 | 0.96 |
| | Complaint | 0 | 1 | 0 | 0.46 |
| | Patient attendants | 1 | 2 | 1 | 0.26 |
| | Patient attendant's age | 23 | 50 | 33 | 6.23 |
| | Educational status of the patient attendants | 0 | 4 | 3 | 1.01 |

Min: Minimum, Max: Maximum, SD: Standard deviation

the preoperative and postoperative AIE and STAI scores of each group, a statistically significant difference was observed between these values for each group (p<0.001). Based on these findings, preoperative AIE and STAI values were found to be significantly higher in both groups when compared with the postoperative period (Table 7).

Additionally, a correlation test was applied for the demographic data and the test results for each group. In the control group, a positive correlation was observed between the PAED and FS scores; between the preoperative AIE scores and preoperative STAI scores and the postoperative AIE scores; and between the preoperative STAI scores, MYPAS scores and the postoperative STAI scores. A negative correlation was observed between the postoperative AIE scores and the educational status of the patient attendants, and between the age of the child and the MYPAS scores. After a correlation analysis of the visual group's findings, a negative correlation was observed between the age of the child to be operated upon and their PAED and MYPAS scores, while a positive correlation was observed between the preoperative and postoperative AIE scores; and between the preoperative and postoperative STAI scores of the patient attendants included in this group. Moreover, a positive correlation was identified between the MYPAS scores of the children and the pre- and postoperative STAI scores of the patient attendants.

**Table 3.** Pediatric Anesthesia Emergence Delirium (PAED) Scale

| Item | Behavior assessed | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| 1 | The child is aware of surroundings | Always | Most of the time | Sometimes | Rarely | Not at all |
| 2 | The child recognizes caregivers | Always | Most of the time | Sometimes | Rarely | Not at all |
| 3 | The child is restless or agitated | Not at all | Rarely | Sometimes | Often | Extremely |
| 4 | The child is crying | Not at all | Rarely | Sometimes | Often | Extremely |
| 5 | The child is inconsolable | Not at all | Rarely | Sometimes | Often | Extremely |

**Table 5.** A descriptive table of the results of the scales applied to children and patient attendants in groups

| Group | Variable | Minimum | Maximum | Median | SD |
|---|---|---|---|---|---|
| Control | PAED | 0 | 20 | 9 | 5.42 |
| | FS0 | 0 | 10 | 4 | 2.60 |
| | FS6 | 0 | 2 | 0 | 0.28 |
| | mYPAS1 | 1 | 4 | 2 | 1.07 |
| | mYPAS2 | 1 | 6 | 2 | 1.72 |
| | mYPAS3 | 1 | 4 | 2 | 1.12 |
| | mYPAS4 | 1 | 4 | 3 | 1.04 |
| | mYPAS5 | 1 | 4 | 2 | 1.08 |
| | AIE-PRE | 14 | 40 | 22 | 5.85 |
| | AIE-POST | 11 | 24 | 14 | 2.60 |
| | STAI-PRE | 31 | 74 | 59 | 10.29 |
| | STAI-POST | 20 | 51 | 30 | 7.61 |
| Visual | PAED | 0 | 18 | 8 | 4.79 |
| | FS0 | 0 | 10 | 4 | 2.19 |
| | FS6 | 0 | 2 | 0 | 0.26 |
| | mYPAS1 | 1 | 4 | 1 | 0.82 |
| | mYPAS2 | 1 | 6 | 1 | 1.16 |
| | mYPAS3 | 1 | 4 | 1 | 0.91 |
| | mYPAS4 | 1 | 4 | 2 | 0.91 |
| | mYPAS5 | 1 | 4 | 1 | 0.82 |
| | AIE-PRE | 11 | 32 | 18 | 4.42 |
| | AIE-POST | 11 | 27 | 13 | 2.89 |
| | STAI-PRE | 21 | 75 | 52 | 10.18 |
| | STAI-POST | 20 | 44 | 27 | 6.49 |

FS: Facial Expression Scale, mYPAS: Yale Preoperative Anxiety Scale, PAED: Pediatric Anesthesia Emergence Delirium, STAI: State-Trait Anxiety Inventory, AIE: Anxiety Regarding Anesthesia, PRE: Preoperative, POST: Postoperative, SD: Standart deviation

**Table 6.** Comparison of preoperative anxiety and postoperative behavioral outcomes between groups

| Variable | Z | p |
|---|---|---|
| mYPAS1 | -3.079 | 0.002 |
| mYPAS2 | -3.498 | <0.001 |
| mYPAS3 | -3.242 | 0.001 |
| mYPAS4 | -3.006 | 0.003 |
| mYPAS5 | -3.620 | <0.001 |
| AIE-PRE | -3.659 | <0.001 |
| AIE-POST | -2.091 | 0.037 |
| STAI-PRE | -3.145 | 0.002 |
| Complaint | -3.767 | <0.001 |

AIE: Anxiety Regarding Anesthesia, mYPAS: Yale Preoperative Anxiety Scale; STAI: State-Trait Anxiety Inventory, PRE: Preoperative, POST: Postoperative, Z: Z score

**Table 7.** Comparison of preoperative and postoperative anxiety levels of patient attendants

| Group | Variable | Z | p |
|---|---|---|---|
| Control | AIE-POST–AIE-PRE | -5.975 | <0.001 |
| | STAI-POST – STAI-PRE | -5.960 | <0.001 |
| Visual | AIE-POST – AIE-PRE | -6.100 | <0.001 |
| | STAI-POST – STAI-PRE | -6.323 | <0.001 |

AIE: Anxiety Regarding Anesthesia, STAI: State-Trait Anxiety Inventory, PRE: Preoperative, POST: Postoperative, Z: Z score

## DISCUSSION

Previous literature has reported higher stress and anxiety levels in young children who underwent surgical interventions than in older children.[14] Young children worry about feeling pain, the possibility of losing body function or possible changes in appearance. They also develop feelings of abandonment and may feel unloved after surgery, which is performed in an unfamiliar environment, and some children even believe that they have been left in the hospital due to bad behavior.[15,16]

It has been demonstrated that children who experience high levels of preoperative anxiety report significantly higher postoperative pain, have delayed hospital discharge, and more frequently exhibit emergence delirium, sleep disturbances, and other maladaptive behavioral changes that may persist for several weeks after surgery.[17]

At the end of our study, the preoperative anxiety levels of the children in the group to whom the visual information form was given were found to decrease significantly. No correlation was observed between the sex and the preoperative anxiety level. The negative behavior of these children in the postoperative period was found to be significantly decreased.

In our study, the preoperative levels of anxiety and delirium in both groups were found to increase as the age decreased, and there was no statistical difference between the PAED scores of the study groups, although preoperative anxiety levels (all subscales of mYPAS) were observed to be lower in the visual group. Based on these findings, providing information through a visual information leaflet could also be effective in young children, and the preoperative concerns of the patient attendants of these children were found to be significantly reduced in the visual group when compared to the control group, and this concern was determined to be less in the postoperative period in the visual group. Compared to the control group, preoperative baseline anxiety levels were found to be lower in the patient attendants to whom the visual information leaflet was given. In our study, however, preoperative anxiety levels were observed to be higher in the patient attendants with a low level of education in the control group. Concerns about the surgery were found to be high in cases where the baseline anxiety was high in the patient attendants, and this concern was observed to continue in the postoperative period. The anxiety levels of patient attendants were also found to increase if the preoperative anxiety level of the child was high. In addition to this, the preoperative level of anxiety and the baseline anxiety level of the patient attendants who were informed with a visual information leaflet were observed to be also higher in the postoperative period if the levels were high before the surgery. When the results of our study were examined, the anxiety levels of patient attendants who were informed with the visual information form were found to be significantly lower than the patient attendants in the control group. However, compatible with literature, a history of previous operations was seen to have no effect on the anxiety levels of the patient attendants or children in our study.[18,19] In accordance with these findings, we believe that providing information through a visual information form is effective in reducing the level of concern and anxiety related

to surgery in both the patient to be operated upon and his or her patient attendants. Although studies have shown fewer complaints of pain in patients, who were informed preoperatively,[20] No statistically significant difference was identified between the groups in the postoperative pain scores in our study. In the light of this finding, the way patients are informed was determined to have no effect on reducing the pain that may occur in the postoperative period.

In conclusion, a visual information form that was designed and used for the first time in our clinic was determined to reduce preoperative anxiety and postoperative agitation levels in children, while also decreasing the concerns of the patient attendants related to preoperative and postoperative anxiety and postoperative anesthesia.

The brochure used in this study enhanced the preoperative evaluation process for pediatric patients by making it more engaging and reassuring. A notable reduction in parental anxiety was observed, which appeared to positively influence children's emotional responses during the preoperative period. The establishment of a dedicated premedication room further supported this approach by allowing pediatric patients and their parents to remain together in a more comfortable environment. Consistent with these observations, highly positive feedback was received from parents in the postoperative period, underscoring the practical value of this intervention in routine clinical practice. In this context, visual information brochures represent a simple, low-cost, and non-pharmacological adjunct that may support perioperative anxiety management in pediatric anesthesia. Nevertheless, further multicenter studies with larger sample sizes and standardized visual tools are needed to confirm these findings and to better define their clinical applicability.

### Summary of Result

In this study, statistically significant differences were observed between the visual and control groups in preoperative and postoperative AIE scores and preoperative State–Trait Anxiety Inventory (STAI) scores. In addition, the incidence of postoperative complications was significantly lower in the visual group.

No statistically significant differences were found between the groups with respect to postoperative STAI, PAED, FES, and mYPAS scores.

Correlation analysis revealed significant associations between mYPAS and FES scores at postoperative minute 0, preoperative AIE and preoperative STAI scores, and mYPAS and postoperative STAI scores.

Furthermore, patient and parental age, history of previous surgery, patient sex, number of siblings, and birth order were not found to have a significant effect on preoperative or postoperative anxiety levels.

### Limitations

This study has several limitations and the results should be interpreted within this context. First, the relatively small sample size, single-center design, and heterogeneity of the surgical procedures may limit the generalizability of the findings to different clinical settings and patient populations. Second, although randomization was implemented, the lack of detailed reporting regarding allocation concealment and assessor blinding may have increased the risk of observer bias. Third, all pediatric patients in the study routinely received midazolam premedication. While this approach is considered appropriate from both ethical and literature-based perspectives, it may have influenced postoperative behavioral outcomes and potentially attenuated the isolated effect of the visual information brochure on anxiety and behavioral measures. In addition, the visual information brochure used in this study was developed locally to meet the specific needs of our clinic and was not externally validated, which may limit its applicability across different clinical settings and patient populations.

In addition, the use of subjective anxiety assessment tools and differences in educational level and sociocultural background of patient attendants may have influenced the study outcomes.

## CONCLUSION

As a result, using a standard information form together with a leaflet that describe the anesthesia application with pictures can be said to reduce anxiety levels and concerns regarding the anesthesia in both the child patient and his/her patient attendants, and may also reduce the rate of negative behaviors observed in children in the postoperative period.

## ETHICAL DECLARATIONS

### Ethics Committee Approval

The study was carried out with the permission of the Kırıkkale University Faculty of Medicine Hospital Scientific Researches Evaluation and Ethics Committee (Date: 19.04.2016, Decision No: 11/02).

### Informed Consent

Informed consent was obtained from a parent or legal guardian. Where appropriate, age-adjusted assent was also obtained from the child. The inclusion of vulnerable populations in this study adhered to national and international ethical guidelines. Extra care was taken to ensure voluntary participation, understanding, and protection of participant dignity and autonomy.

### Peer Review Process

This manuscript was subject to external peer review.

### Conflict of Interest

The authors declare no conflicts of interest related to this study.

### Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

### Author Contributions

Concept: ZBG, Design: ZBG, Control: GA, IG, ÜB, Resources: ZBG, EP, Materials: ZBG, Data Collection: ZBG, Analysis: IG, ÜB, Literature Review: ZBG, EP, Writing: ZBG, EP, Critical Review: IG, GA,ÜB.

## REFERENCES

1. Ahmadipour M, Sattari H, Nejad MA. Incidence and risk factors related to anxiety of children and adolescents before elective surgery. *Eur J Transl Myol*. 2022;32(2):10449. doi:10.4081/EJTM.2022.10449
2. Derlemesi S, Akkoyun S. The effect of nonpharmacological methods used in the preoperative period on anxiety levels in pediatric surgery patients: a systematic review of postgraduate theses. *J Curr Nurs Res*. 2025;5(2):141-158.
3. Santapuram P, Stone AL, Walden RL, Alexander L. Interventions for parental anxiety in preparation for pediatric surgery: a narrative review. *Children*. 2021;8(11):1069. doi:10.3390/CHILDREN8111069
4. Ma X, Zhang Z, Bao Y, Zhao H. Impact of pediatric surgery on anxiety in children and their families and coping strategies: a narrative review. *Transl Pediatr*. 2025;14(4):718-727. doi:10.21037/TP-2025-10/COIF
5. Meletti DP, Meletti JFA, Camargo RPS, Silva LM, Módolo NSP. Psychological preparation reduces preoperative anxiety in children. Randomized and double-blind trial. *J Pediatr (Rio J)*. 2019;95(5):545-551. doi:10.1016/j.jped.2018.05.009
6. Güvenç H, Bilgili YD, Özçınar Orhan S, et al. Perioperatif anksiyetenin giderilmesinde görsel bilgilendirme teknolojisinin yeri. *Turkish J Pediatr Dis*. 2022;16(4):326-331. doi:10.12956/TCHD.1002732
7. Kvaal K, Ulstein I, Nordhus IH, Engedal K. The Spielberger State-Trait Anxiety Inventory (STAI): the state scale in detecting mental disorders in geriatric patients. *Int J Geriatr Psychiatry*. 2005;20(7):629-634. doi:10.1002/GPS.1330
8. Marteau TM, Bekker H. The development of a six-item short-form of the state scale of the Spielberger State—Trait Anxiety Inventory (STAI). *British J Clin Psychol*. 1992;31(3):301-306. doi:10.1111/J.2044-8260.1992.TB00997.X
9. Hume MA, Kennedy B, Asbury AJ. Patient knowledge of anaesthesia and peri-operative care. *Anaesthesia*. 1994;49(8):715-718. doi:10.1111/J.1365-2044.1994.TB04408.X
10. Prayunanto E, Widyastuti Y, Sari D. The Association of Modified Yale Perioperative Anxiety Scale and pediatric anesthesia behavior on postoperative emergence delirium in children: a prospective cohort study. *Bali J Anesthesiol*. 2023;7(2):88-93. doi:10.4103/BJOA.BJOA_22_23
11. Hatipoğlu Z, Kirdök O, Özcengiz D. Validity and reliability of the Turkish version of the modified Yale Preoperative Anxiety Scale. *Turk J Med Sci*. 2019;49(3):730. doi:10.3906/SAG-1612-113
12. Sudhakar Russell PS, Mammen PM, Shankar SR, et al. Pediatric Anesthesia Emergence Delirium Scale: a diagnostic meta-analysis. *World J Clin Pediatr*. 2022;11(2):196. doi:10.5409/WJCP.V11.I2.196
13. Drendel AL, Kelly BT, Ali S. Pain assessment for children: overcoming challenges and optimizing care. *Pediatr Emerg Care*. 2011;27(8):773-781. doi:10.1097/PEC.0B013E31822877F7
14. Ma X, Zhang Z, Bao Y, Zhao H. Impact of pediatric surgery on anxiety in children and their families and coping strategies: a narrative review. *Transl Pediatr*. 2025;14(4):718-727. doi:10.21037/TP-2025-10/PRF
15. Liang Y, Huang W, Hu X, et al. Preoperative anxiety in children aged 2–7 years old: a cross-sectional analysis of the associated risk factors. *Transl Pediatr*. 2021;10(8):2024034-2022034. doi:10.21037/TP-21-215
16. Liu W, Xu R, Jia J, Shen Y, Li W, Bo L. Research progress on risk factors of preoperative anxiety in children: a scoping review. *Int J Environ Res Public Health*. 2022;19(16):9828. doi:10.3390/IJERPH19169828
17. Fortier MA, Kain ZN. Treating perioperative anxiety and pain in children: a tailored and innovative approach. *Paediatr Anaesth*. 2014;25(1):27. doi:10.1111/PAN.12546
18. Topalel S, Orekici Temel G, Azizoğlu M. Evaluation of preoperative anxiety in Turkish paediatric patients and validity and reliability of the Turkish Modified Yale Preoperative Anxiety Scale. *Turk J Anaesthesiol Reanim*. 2020;48(6):484. doi:10.5152/TJAR.2020.116
19. Caumo W, Schmidt AP, Schneider CN, et al. Risk factors for preoperative anxiety in adults. *Acta Anaesthesiol Scand*. 2001;45(3):298-307. doi:10.1034/J.1399-6576.2001.045003298.X
20. Darville-Beneby R, Lomanowska AM, Yu HC, et al. The impact of preoperative patient education on postoperative pain, opioid use, and psychological outcomes: a narrative review. *Canadian J Pain*. 2023;7(2):2266751. doi:10.1080/24740527.2023.2266751

Ankyra Medical Journal
Original Article
DOI: 10.51271/ANKMJ-0064

# Effects of commercially available toothpastes on the surface roughness of a universal nanohybrid composite resin: a profilometric study

Cansu Yıkıcı Çöl*[1], Sema Yazıcı Akbıyık[2]

[1]Department of Restorative Dentistry, Panorama Ankara Oral and Dental Health Clinic, Ankara, Turkiye
[2]Department of Restorative Dentistry, Faculty of Dentistry, Lokman Hekim University, Ankara, Turkiye

**Cite this article:** Yıkıcı Çöl C, Yazıcı Akbıyık S. Effects of commercially available toothpastes on the surface roughness of a universal nanohybrid composite resin: a profilometric study. *Ank Med J.* 2026;5(2):40-45. doi:10.51271/ANKMJ-0064

**Received**: 29/12/2025 ◆ **Accepted**: 21/01/2026 ◆ **Published**: 25/03/2026

## ABSTRACT

**Aims:** The aim of this in vitro study was to evaluate the effects of different commercially available toothpastes on the surface roughness of a universal nanohybrid composite resin following simulated toothbrushing.

**Methods:** Eighty cylindrical specimens of a universal nanohybrid composite resin were prepared and randomly assigned to ten groups (n=8), including nine toothpaste groups and one control group brushed with distilled water. Surface roughness (Ra, μm) was measured before and after standardized simulated toothbrushing using a contact profilometer. Toothbrushing was performed with an electric toothbrush and toothpaste slurry prepared at a 1:1 ratio. Statistical analysis was conducted using the Wilcoxon Signed-Rank test, Kruskal–Wallis test, and Mann–Whitney U test with Holm–Bonferroni correction (α=0.05).

**Results:** All groups demonstrated statistically significant changes in surface roughness after brushing ($p=0.0078$); however, the magnitude and direction of change varied among the toothpastes. Significant differences in ΔRa values were observed between certain groups ($p<0.05$). Among the tested toothpastes, the ROC group was the only group that exhibited a reduction in surface roughness following brushing.

**Conclusion:** Different toothpastes produced varying effects on the surface roughness of nanohybrid composite resin. Although some whitening and herbal toothpastes increased surface roughness, the changes generally remained within clinically acceptable limits. These findings highlight the importance of toothpaste selection for patients with composite resin restorations.

**Keywords:** Activated charcoal, dentifrice, resin composite, surface roughness, toothpaste, whitening

## INTRODUCTION

Composite resin materials play a fundamental role in contemporary restorative dentistry by providing an optimal combination of esthetic appeal, functional performance, and clinical versatility.[1] However, the long-term clinical success of composite resin restorations is closely related to their ability to maintain surface smoothness.[2]

A smooth restorative surface is essential for achieving optimal esthetic outcomes while minimizing plaque accumulation.[3] In contrast, increased surface irregularities may reduce surface gloss and promote discoloration, thereby compromising the overall esthetic appearance of the restoration.[4] Surface roughness describes the finer irregularities present on a material's surface, which typically arise from its intrinsic properties or the manufacturing process.[5]

Daily oral hygiene procedures, particularly toothbrushing with toothpastes, represent one of the main extrinsic factors affecting the surface integrity of composite resin materials.[6] Toothpastes are composed of a variety of chemical and mechanical agents—including abrasive particles, surfactants, fluoride, and other therapeutic additives that have been reported to produce distinct effects on the surface characteristics of restorative materials.[7] Among these components, abrasive particles play a central role in stain removal but may also contribute to increased surface roughness of composite restorations.[8]

The most commonly used abrasive agents in contemporary toothpastes include hydrated silica, calcium carbonate, dicalcium phosphate dihydrate, and sodium bicarbonate, each exhibiting different abrasive potentials depending on particle size, shape, and concentration. Whitening toothpastes, which have gained popularity due to increasing esthetic demands, primarily act by removing extrinsic stains through mechanical abrasion rather than chemical bleaching.[8]

*Corresponding Author: Cansu Yıkıcı Çöl, cansu.yikici@gmail.com

Several in vitro studies have reported that whitening, charcoal-based toothpastes may increase surface roughness of composite resin materials following simulated toothbrushing.[9-11] The abrasive nature of charcoal-containing dentifrices has raised concerns regarding their potential to increase surface roughness and compromise the integrity of composite restorations.[2] Systematic reviews have reported conflicting findings regarding the effects of toothpastes on the surface roughness of composite resins, highlighting the need for further standardized investigations.[2,12]

Despite the growing number of commercially available toothpastes, limited data exist comparing their abrasive effects on modern universal nanohybrid composite resin materials under standardized brushing conditions. Therefore, the aim of this in vitro study was to evaluate the effect of different commercially available toothpastes on the surface roughness of a universal nanohybrid composite resin following simulated toothbrushing.

The null hypothesis of this study was that different commercially available toothpastes would not cause any significant change in the surface roughness of the universal nanohybrid composite resin.

## METHODS

This study was conducted exclusively on in vitro materials; since no human or animal subjects were used, approval from an ethics committee is not required.

This in vitro research examined how nine different commercially available toothpastes influence the surface roughness of a universal nanohybrid composite resin material when exposed to standardized, simulated toothbrushing procedures. The surface roughness (Ra) of the samples was measured both before and after the brushing simulation using a profilometric analysis system. Their compositions and manufacturers are presented in **Table 1**.

The required sample size was determined using the G*Power software (version 3.1.9.4, Düsseldorf, Germany) with a desired power of 95% and α=0.05, and at least eight samples were obtained for each group. A total of 80 samples were prepared for 10 study groups (n=8). Cylindrical samples (8 mm in diameter and 2 mm in thickness) were fabricated for each material using plastic molds. The materials were placed in a single layer and covered with Mylar strips and glass slides to achieve a uniform surface finish. They were cured for 20

**Table 1.** Composition and manufacturing details of the experimen materials

| Toothpastes | Group code | Compositions | Manufacturers |
|---|---|---|---|
| **Control group** | **C** | **Distilled water** | - |
| Agarta | AGA | Sorbitol, *hydrated silica*, aqua, glycerin, xylitol, lauryl glucoside, cocamidopropyl betaine, zinc oxide, cocos nucifera oil, xantan gum, mentha piperita oil, menthol, eugenia caryophyllus leaf oil, citrus limon peel oil, phenylpropanol, caprylyl glycol, aloe barbadensis leaf extract, rebausioside a, salvia officinalis oil, eucalyptus globulus leaf oil, melameuca alternifolia leaf oil. | Güneyce Chemistry, (Ankara Turkiye) |
| Biomed Charcoal | CHA | Aqua, hydrogenated starch hydrolysate, *dicalcium phosphate dihydrate, hydrated silica*, glycerin, sodium coco-sulfate, cellulose gum, aroma, calcium hydroxyapatite, zinc citrate, benzyl alcohol, *sodium bicarbonate*, tetrasodium glutamate diacetate, xanthan gum, menthol, l-lysine, *charcoal powder*, xylitol, carbon black, cymbopogon flexuosus herb oil, mentha piperita oil, cinnamomum camphora bark oil, sodium benzoate, ananas sativus fruit extract, maltodextrin, cedrus atlantica bark oil, betula alba leaf extract, plantago major leaf extract, potassium sorbate, arginine, sodium hydroxide, helianthus annuus seed oil, limonene, citral. | STS holoding, Gabrova, Bulgaria |
| LR Aloe.via sensitive protect tooth paste | ALO | Aloe barbadensis leaf juice, *calcium carbonate*, glycerin, sorbitol, potassium chloride, silica, sodium monofluorophosphate (1440 ppm), cocamidopropyl betaine, hydroxyethylcellulose, flavor, sodium saccharin, sodium hydroxide, CI 42090 (blue 1), CI19140 (yellow 5) | LR Health & Beauty, Ahlen, Germany) |
| Meridol | MER | Glycerin, aqua, *hydrated silica,* hydroxyethylcellulose, olaflur*, sodium gluconate, propylene glycol, aroma, cocamidopropyl betaine, peg-3 tallow aminopropylamine, caprylyl glycol, stannous chloride, 1440 ppm f, sodium saccharin, phenylpropanol, limonene, CI 74160. | Colgate Palomoliv, Świdnica, Poland |
| Opalescence | OPA | Aqua, *silica*, glycerin, sorbitol, xylitol, aroma (flavor), poloxamer 407, sodium lauryl sulfate, carbomer, sodium benzoat, sodium flüoride (1100 Ppm), sodium hydroxide, sucralose, xantan gum, FD&C, blue no.1(CI 42090), FD&C yellow no.5(CI 19140) | Opalescence (Ultradent Products, South Jordan USA) |
| Oral-B pro-3D white clinical | ORA | Sorbitol, *hydrated silica*, sodium hexametaphosphate, disodium pyrophosphate, sodium lauryl sulfate, carrageenan, aroma, sodium monofluorophosphate (1450 ppm), xanthan gum, sodium saccharin, cocamidopropyl betaine, phosphoric acid, sodium chloride, sucralose, cinnamal, benzyl alcohol, eugenol, sodium hydroxide, sodium benzoate, CI 74160, CI 74260, citric acid, sodium citrate, potassium sorbate | GmbH, Procter &Gamble, Germany |
| R.O.C.S Uno | ROC | Aqua, *silica*, glycerin, xylitol (%2,2), xanthan gum, aroma (flavor), sodium glycerophosphate, magnesium chloride, calcium glycerophosphate, sodium lauryl sulfate (SLS), sodium saccharin, sodium silicate, methylparaben, propylparaben and limonene. | R.O.C.S. Laboratories AG (Switzerland) |
| Sensodyne Nourish | NOU | Aqua, sorbitol, *hydrated silica,* glycerin, potassium nitrate, sodium lauryl sulfate (SLS), flavour (natural mint and citrus oils), xanthan gum, cocamidopropyl betaine, sodium fluoride (≈ 1450 ppm), sodium saccharin, *titanium dioxide*, limonene and benzyl alcohol | Haleon (Weybridge, United Kingdom) |
| Sensodyne Pronamel | PRO | Aqua, sorbitol, *hydrated silica*, glycerin, potassium nitrate, PEG-6, cocamidopropyl betaine, sodium lactate, glaxosmithkline aroma, xanthan gum, sodium flüoride (1450ppm), sodium saccharin, *titanium dioxide*, PVM/MA copolymer, sodium hydroxide, limonene. | Haleon (Weybridge, United Kingdom) |
| Universal, nano-hybrid composite | | *Barium aluminum borosilicate glass, silicon dioxide*, HEDMA, BisGMA, TCDDMA, *Fumed silica,* TEGDMA, BisEMA, initiator, stabilizer, pigment | Grandio SO (VOCO, Cuxhaven, Germany) |

Abrasive components are indicated in ithalic, whereas remineralizing or auxiliary ingredients are underlined in the toothpaste group

seconds with an LED curing device (Woodpecker, Guilin Woodpecker Medical Instrument Co., China) operating at approximately 1200 mW/cm².

To ensure surface standardization, the specimens were sequentially polished with silicon carbide abrasive papers of 600, 800, and 1200 grit under continuous water cooling. Following polishing, the samples were ultrasonically cleaned for 10 minutes to eliminate surface residues and subsequently stored in distilled water at 37°C for 24 hours before surface roughness evaluation. All samples were randomly distributed into 10 groups. Group codes are shown in Table 1.

Each specimen was brushed for a total duration of 4 minutes, simulating approximately one month of clinical toothbrushing, in accordance with the protocol described by Gömleksiz and Okumuş,[13] using an electric toothbrush (Oral-B iO, Braun, Germany) equipped with medium-hardness bristles . The toothpaste slurry was prepared at a 1:1 toothpaste-water ratio, as this proportion has been widely adopted in previous in vitro toothbrushing studies[14,15] to simulate the dilution of toothpaste by saliva during clinical brushing conditions. For the control group, brushing was performed using distilled water alone for the same brushing duration. Following the brushing procedure, all specimens were rinsed under running tap water and subsequently stored at 37°C for 24 hours prior to the final surface roughness measurements.

Surface roughness (Ra, µm) was evaluated both before and after the brushing procedure using a contact profilometer[9] (Perthometer; Mahr GmbH, Ingolstadt, Germany). For each specimen, measurements were taken at three separate locations, and the average Ra value was calculated and used for statistical analysis. The difference between pre and post-brushing Ra values was determined to assess changes in surface roughness.

### Statistical Analysis

The data analyses were performed using IBM SPSS Statistics software (version 26, IBM Corp., Armonk, NY, USA). Descriptive data were summarized as before, after, and delta median [interquartile range, (IQR)] values. The Shapiro–Wilk test was used to evaluate the normality of the data distribution. As the assumptions of normality were not met ($p<0.05$), non-parametric statistical methods were applied. Within-group comparisons between before and after brushing measurements were conducted using the Wilcoxon Signed-Rank test. Intergroup comparisons were performed using the Kruskal–Wallis H test, and when significant differences were observed, post hoc pairwise comparisons were performed using the Mann–Whitney U test with Holm-Bonferroni correction. A significance level of $p<0.05$ was adopted for all statistical evaluations.

## RESULTS

All analyses were performed on the original assigned groups, with 8 specimens included in each group. Table 2 presents descriptive statistics including the before, after brushing median surface roughness (Ra, µm (IQR)) and ΔFinal-Initial [IQR]), values of the test groups. Within-group comparisons revealed statistically significant differences between pre- and post-brushing surface roughness values for all groups according to the Wilcoxon signed-rank test ($p=0.0078$) (Table 2). However, the magnitude and direction of surface roughness changes varied among the groups, as reflected by the ΔRa values.

**Table 2.** Descriptive statistics of the study

| | Initial (median (IQR)) | After (median (IQR)) | Δ Final-initial (IQR) | p |
|---|---|---|---|---|
| C | 0.26 (0.013) | 0.23 (0.054) | -0.019(0.050) | 0.0078 |
| AGA | 0.24 (0.019) | 0.28 (0.026) | 0.050(0.031) | |
| CHA | 0.23 (0.025) | 0.29 (0.029) | 0.058 (0.035) | |
| ALO | 0.24 (0.017) | 0.28 (0.026) | 0.046 (0.028) | |
| MER | 0.24 (0.023) | 0.29 (0.032) | 0.052 (0.034) | |
| OPA | 0.24 (0.023) | 0.29 (0.028) | 0.045 (0.028) | |
| ORA | 0.24 (0.022) | 0.29 (0.030) | 0.050 (0.029) | |
| ROC | 0.24 (0.019) | 0.22 (0.008) | -0.014 (0.017) | |
| NOU | 0.23 (0.021) | 0.28 (0.026) | 0.010 (0.026) | |
| PRO | 0.23 (0.020) | 0.28 (0.025) | 0.050 (0.025) | |

C: Control, AGA: Agarta, CHA: Biomed Charcoal, ALO: LR Aloe.Via sensitive protect tooth paste, MER: Meridol, OPA: Opalescence, ORA: Oral-B pro-3D white clinical, ROC: R.O.C.S Uno, NOU: Sensodyne Nourish, PRO: Sensodyne Pronamel

The Kruskal–Wallis test revealed a statistically significant difference among the groups in terms of ΔRa values ($H=78.22$, $p<0.001$, $df=9$). Post hoc pairwise comparisons performed using the Mann–Whitney U test with Holm–Bonferroni correction demonstrated that statistically significant differences in ΔRa values were observed in a limited number of group comparisons ($p<0.05$) (Table 3). The ROC group differed significantly from several toothpaste groups, including AGA, ALO, MER, OPA, ORA, and PRO.

In addition, significant differences were detected between the control group and some toothpaste groups, such as ALO, MER, OPA, ORA, and PRO. Significant differences were observed between the PRO group and the AGA, ALO, MER, and NOU groups. Conversely, the majority of comparisons among conventional toothpaste groups did not reach statistical significance after Holm–Bonferroni correction ($p>0.05$), suggesting similar effects on surface roughness change among these products (Table 3).

## DISCUSSION

The null hypothesis of the present study, which stated that different commercially available toothpastes would not affect the surface roughness of a universal nanohybrid composite resin, was rejected. The results demonstrated statistically significant differences in surface roughness changes among the toothpaste groups, as revealed by the Kruskal–Wallis test ($H=78.22$, $p<0.001$). These findings indicate that the type of toothpaste used can significantly influence the surface characteristics of composite resin restorations.

The Ra value is the main parameter to describe the surface roughness of a flattened surface. Surface roughness is clinically significant because roughened surfaces promote bacterial adhesion, staining, and reduced gloss, affecting both esthetics and restoration longevity. A threshold of approximately 0.2 µm has been identified as critical for plaque retention, meaning even small increases in surface irregularity can contribute to biofilm accumulation and degradation of restorative margins. This threshold is well-documented, as "an average roughness greater than 0.2

**Table 3.** Pairwise comparisons using the Mann–Whitney U test with Holm–Bonferroni correction

| Comparison | Holm-adjusted p-value | Comparison | Holm-adjusted p-value | Comparison | Holm-adjusted p-value |
|---|---|---|---|---|---|
| C &AGA | 0.076768 | AGA & NOU | 0.310023 | MER & OPA | 1.000000 |
| C &CHA | 0.798135 | AGA & PRO | **0.006993** | MER & ORA | 0.506294 |
| C & ALO | **0.031546** | CHA & ALO | 1.000000 | MER & ROC | **0.006993** |
| C & MER | **0.018648** | CHA & MER | 0.909402 | MER & NOU | 0.052214 |
| C & OPA | **0.011189** | CHA & OPA | 0.413364 | MER & PRO | **0.011189** |
| C & ORA | **0.011189** | CHA & ORA | 0.413364 | OPA & ORA | 1.000000 |
| C & ROC | 1.000000 | CHA & ROC | 0.909402 | OPA & ROC | **0.006993** |
| C & NOU | 1.000000 | CHA & NOU | 1.000000 | OPA & NOU | **0.011189** |
| C & PRO | **0.006993** | CHA & PRO | 0.076768 | OPA & PRO | 0.644600 |
| AGA & CHA | 1.000000 | ALO & MER | 1.000000 | ORA & ROC | **0.006993** |
| AGA & ALO | 1.000000 | ALO & OPA | 0.798135 | ORA & NOU | 0.011189 |
| AGA & MER | 0.909402 | ALO & ORA | 0.153846 | ORA & PRO | 1.000000 |
| AGA & OPA | 0.076768 | ALO & ROC | **0.006993** | ROC & NOU | 1.000000 |
| AGA & ORA | 0.107226 | ALO & NOU | 0.052214 | ROC & PRO | **0.006993** |
| AGA & ROC | **0.006993** | ALO & PRO | **0.011189** | NOU & PRO | **0.006993** |

Statistically significant ($p<0.05$) comparisons are indicated in bold. C: Control, AGA: Agarta, CHA: Biomed Charcoal, ALO: LR Aloe.Via sensitive protect tooth paste, MER: Meridol, OPA: Opalescence, ORA: Oral-B pro-3D white clinical, ROC: R.O.C.S Uno, NOU: Sensodyne Nourish, PRO: Sensodyne Pronamel

µm is associated with a substantial increase in bacterial retention".[16,17] In the present study, although statistically significant changes in surface roughness were observed, all ΔRa values remained below the 0.2 µm plaque-retention threshold, indicating that the observed changes may have limited clinical relevance in terms of plaque accumulation.

The nanohybrid composite used in the study contains a resin matrix in which BisGMA-based formulations are characterized by high viscosity due to strong hydrogen bonding, leading to reduced polymerization shrinkage and improved wear resistance and mechanical strength; however, this high viscosity often necessitates the incorporation of diluent monomers. BisEMA, with its ethoxylated structure and lower viscosity compared with BisGMA, improves handling and flow while maintaining acceptable mechanical durability, though its lower hydrogen bonding may slightly compromise wear resistance when used alone. TEGDMA, a low-molecular-weight diluent monomer, significantly reduces viscosity and enhances degree of conversion, but its flexible structure increases polymer network mobility, which has been associated with higher wear rates and reduced long-term mechanical stability, especially under cyclic loading. In contrast, TCDDMA and HEDMA are more rigid aliphatic dimethacrylates that increase cross-link density, leading to improved hardness, wear resistance, and resistance to mechanical degradation, while maintaining lower viscosity than BisGMA-dominant systems. Overall, the balance between these monomers strongly influences clinical performance: formulations richer in BisGMA, TCDDMA, or HEDMA tend to show superior wear resistance and durability, whereas increased TEGDMA or BisEMA content enhances handling and viscosity at the expense of long-term mechanical stability, underscoring the importance of optimized monomer combinations rather than reliance on a single resin component.[18]

Many studies have evaluated how whitening toothpastes influence the surface roughness of restorative materials. Roopa et al.[19] as well as Dayı and Öcal,[20] reported that whitening toothpastes resulted in a statistically significant increase in the surface roughness of composite materials. Yılmaz et al.[21] concluded that the observed whitening effect is mainly attributable to the brushing process itself rather than the specific composition of the toothpaste. In line with this finding, da Rosa et al.[22] showed that the use of whitening toothpastes did not cause a significant increase in the surface roughness of nanohybrid composite resin after a one-month simulated brushing period. Manis et al.[23] observed a decrease in Ra values in simulated toothbrushing using nanohybrid composite resin, regardless of the toothpaste used.

The results showed that among the groups marketed as whitening toothpastes (CHA, OPA, ORA, ROC, and NOU), only the OPA and ORA groups exhibited statistically significantly higher surface roughness values compared with the control group. The ROC group was the only group, apart from the control group, that demonstrated lower median surface roughness values after brushing than before brushing. The ROC group also showed the most pronounced difference among the AGA, ALO, MER, OPA, ORA, and PRO groups, presenting a distinct surface roughness pattern with statistically significantly lower values. The findings of this study indicate that whitening toothpastes did not produce a consistent surface roughness pattern on nanohybrid composite resins, although variations in surface roughness among the groups were observed depending on the toothpaste used.

Polan and Gürgan[11] (2-week brushing period), Koç Vural et al.[24] (12-week brushing period) investigated the effects of activated charcoal-containing whitening toothpastes on the surface roughness of composite resins and showed that it caused a statistically significant increase in surface roughness, similar to the findings of the present study (CHA ΔRa 0.058, $p=0.0078$). In contrast, no significant change in surface roughness was observed in the control group brushed only with distilled water. The study also showed that the increase in surface roughness was not solely attributable to the presence of activated charcoal but was also influenced by

other abrasive components in the toothpaste formulations, such as hydrated silica, silica, mica, as well as particle characteristics including size, shape, and hardness. In the present study, no statistically significant differences were observed among any of the toothpaste groups, including the activated charcoal–containing toothpaste and the control group.

Herbal and non-herbal toothpastes demonstrated an increase in enamel surface roughness within clinically acceptable limits.[25] Following six months of simulated toothbrushing, two different herbal toothpastes produced a statistically significant increase in surface roughness on nanofilled composite surfaces. This finding has been attributed to the detachment of filler particles from the composite matrix during the abrasion process. The results of the study indicate that herbal toothpastes may adversely affect the surface texture of restorative materials and that their long-term use may lead to increased plaque accumulation and aesthetic deterioration as a consequence of elevated surface roughness.[26] In the present study, the groups classified under the herbal toothpaste category, AGA and ALO, exhibited significantly higher post-brushing surface roughness values compared with their baseline measurements (AGA $\Delta Ra=0.050$, $p=0.0078 <0.05$; ALO $\Delta Ra=0.046$, $p=0.0078 <0.05$). Intergroup comparisons revealed statistically significant differences only between the ROC and PRO groups ($p<0.05$).

### Limitations

This study has several limitations that should be considered when interpreting the results. First, the investigation was conducted under in vitro conditions, which may not fully replicate the complex oral environment, where factors such as saliva composition, temperature fluctuations, pH changes, dietary habits, and masticatory forces can influence the wear behavior of restorative materials. Second, artificial aging procedures, such as thermocycling or long-term water storage, were not applied prior to or during the brushing simulation; therefore, the potential effects of material aging on surface roughness could not be assessed. Additionally, only one universal nanohybrid composite resin with a specific resin matrix composition was evaluated, which limits the generalizability of the findings to other composite types with different filler sizes, filler loadings, or resin formulations. Furthermore, the RDA values of the tested toothpastes could not be considered, as these data are not consistently disclosed by manufacturers, limiting direct correlation between surface roughness changes and standardized abrasivity indices. Finally, the brushing protocol was standardized using a single electric toothbrush type and a fixed brushing duration, which may not fully represent the variability in brushing techniques, forces, and durations observed in daily clinical practice.

## CONCLUSION

Future studies should incorporate artificial aging protocols, such as thermocycling and prolonged water storage, to better simulate long-term clinical conditions and evaluate their combined effects of toothbrushing on composite resin surface roughness. The use of composite resins with different resin matrices, filler morphologies, and filler loadings—including microhybrid, nanofilled, bulk-fill, and flowable composites—would provide a broader understanding of material-dependent surface changes. Moreover, extending the brushing duration to simulate longer periods of clinical use may help clarify cumulative abrasive effects over time. Comparative evaluations using both electric and manual toothbrushes, as well as varying brushing forces and techniques, would further enhance the clinical relevance of future investigations. In addition, in vivo or long-term clinical studies are warranted to confirm the in vitro findings and to assess the impact of toothpaste-induced surface roughness changes on plaque accumulation, discoloration, and restoration longevity.

## ETHICAL DECLARATIONS

### Ethics Committee Approval

This study was conducted exclusively on in vitro materials; since no human or animal subjects were used, approval from an ethics committee is not required.

### Informed Consent

This study was conducted exclusively on in vitro materials; since no human or animal subjects were used, informed consent is not required.

### Peer Review Process

This manuscript was subject to external peer review.

### Conflict of Interest

The authors declare no conflicts of interest related to this study.

### Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

### Author Contributions

Concept: CYÇ; Design: CYÇ; Control: CYÇ, SYA; Resources: CYÇ, SYA; Materials: CYÇ, SYA; Data Collection and/or Processing: CYÇ, SYA; Analysis and/or Interpretation: CYÇ, SYA; Literature Review: CYÇ, SYA; Writing the Article: CYÇ, SYA; Critical Review: CYÇ, SYA.

## REFERENCES

1. Raghu R, Srinivasan R. Optimizing tooth form with direct posterior composite restorations. *J Conserv Dent.* 2011;14(4):330-336. doi:10.4103/0972-0707.87192
2. Wiktorski CA, Michelogiannakis D, Rossouw PE, Javed F. The effect of charcoal-based dentifrice and conventional whitening toothpaste on the color stability and surface roughness of composite resin: a systematic review of in vitro studies. *Dent J.* 2024;12(3):58. doi:10.3390/dj12030058
3. Hotta M, Hirukawa H, Aono M. The effect of glaze on restorative glass-ionomer cements. *J Oral Rehabil.* 1995;22(3):197-201. doi:10.1111/j.1365-2842.1995.tb01564.x
4. Sakaguchi R, Douglas WH, DeLong R, Pintado M. The wear of a posterior composite in an artificial mouth: a clinical correlation. *Dent Mater.* 1986;2(6):235-240. doi:10.1016/s0109-5641(86)80034-3
5. Paravina RD, Powers JM. Esthetic color training in dentistry. St. Louis, MO: Elsevier Mosby; 2004.
6. Roselino LdMR, Chinelatti MA, Alandia-Román CC, Pires-de-Souza FdCP. Effect of brushing time and dentifrice abrasiveness on color change and surface roughness of resin composites. *Braz Dent J.* 2015;26(5):507-513. doi:10.1590/0103-6440201300399

7. Carvalho LF, Alves LMM, Bergamo ET, et al. Influence of abrasive dentifrices on polymeric reconstructive material properties after simulated toothbrushing. *Biomater Investig Dent.* 2023;10(1):2268670. doi:10.1080/26415275.2023.2268670
8. Joiner A. Whitening toothpastes: a review of the literature. *J Dent.* 2010; 38(Suppl 2):e17-e24. doi:10.1016/j.jdent.2010.05.017
9. Ferreira NMR, Lippert VF, Heck ABdS, et al. Surface roughness of composite resins subjected to brushing with whitening toothpastes: an in vitro study. *Braz Oral Res.* 2025;39:e006. doi:10.1590/1807-3107bor-2025.vol39.006
10. Uppal M, Ganesh A, Balagopal S, Kaur G. Profilometric analysis of two composite resins' surface repolished after tooth brush abrasion with three polishing systems. *J Conserv Dent.* 2013;16(4):309-313. doi:10.4103/0972-0707.114356
11. Polan MN, Gurgan S. Effects of different charcoal-containing whitening toothpastes on color and surface roughness of a supra-nanofilled composite resin. *BMC Oral Health.* 2025;25(1):1083. doi:10.1186/s12903-025-06401-8
12. Jamwal N, Rao A, Shenoy R, Pai M, Ks A, Br A. Effect of whitening toothpaste on surface roughness and microhardness of human teeth: a systematic review and meta-analysis. *F1000Res.* 2022;11:22. doi:10.12688/f1000research.76180.3
13. Gömleksiz S, Okumuş ÖF. The effect of whitening toothpastes on the color stability and surface roughness of stained resin composite. *BMC Oral Health.* 2024;24(1):860. doi:10.1186/s12903-024-04654-3
14. Aydın N, Karaoglanoglu S, Oktay EA. Investigation the effects of whitening toothpastes on color change of resin-based CAD/CAM blocks. *J Esthet Restor Dent.* 2021;33(6):884-890. doi:10.1111/jerd.12627
15. Enax J, Fandrich P, Schulze zur Wiesche E, Amaechi BT. The whitening efficacy of a hydroxyapatite toothpaste and a blue covarine toothpaste: a comparative in vitro study. *Dent J (Basel).* 2025;13(4):143. doi:10.3390/dj13040143
16. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dent Mater.* 1997; 13(4):258-269. doi:10.1016/s0109-5641(97)80038-3
17. Zhang K, Zhang N, Weir MD, Reynolds MA, Bai Y, Xu HH. Bioactive dental composites and bonding agents having remineralizing and antibacterial characteristics. *Dent Clin North Am.* 2017;61(4):669-687. doi:10.1016/j.cden.2017.05.002
18. Dantagnan C-A, Babajko S, Nassif A, et al. Analysis of resin-based dental materials' composition depending on their clinical applications. *Polymers.* 2024;16(8):1022. doi:10.3390/polym16081022
19. Roopa K, Basappa N, Prabhakar A, Raju O, Lamba G. Effect of whitening dentifrice on micro hardness, colour stability and surface roughness of aesthetic restorative materials. *J Clin Diagn Res.* 2016;10(3):ZC06-ZC11. doi:10.7860/JCDR/2016/15700.7350
20. Dayi B, Ocal F. The effect of whitening toothpastes on colour change and surface roughness of restorative materials. *Odovtos Int J Dent Sci.* 2023;25(2):40-52. doi:10.15517/ijds.2022.52728
21. Yilmaz MN, Gul P, Unal M, Turgut G. Effects of whitening toothpastes on the esthetic properties and surface roughness of a composite resin. *J Oral Sci.* 2021;63(4):320-325. doi:10.2334/josnusd.21-0044
22. da Rosa GM, da Silva LM, de Menezes M, do Vale HF, Regalado DF, Pontes DG. Effect of whitening dentifrices on the surface roughness of a nanohybrid composite resin. *Eur J Dent.* 2016;10(02):170-175. doi:10.4103/1305-7456.178305
23. Manis RB, Da Silva TM, Franco TT, Dantas DCB, Franco LT, Huhtala MFRL. Influence of whitening toothpaste on color, roughness, and microhardness of composite resins. *Eur J Gen Dent.* 2017;6(2):92-98. doi:10.4103/ejgd.ejgd_53_17
24. Koc Vural U, Bagdatli Z, Yilmaz AE, Yalçın Çakır F, Altundaşar E, Gurgan S. Effects of charcoal-based whitening toothpastes on human enamel in terms of color, surface roughness, and microhardness: an in vitro study. *Clin Oral Investig.* 2021;25(10):5977-5985. doi:10.1007/s00784-021-03903-x
25. Kaur R, Dodamani A, Patil S, et al. Comparative analysis of enamel surface roughness between herbal and non-herbal dentifrices: an in vitro study. *Cureus.* 2025;17(3):e80436. doi:10.7759/cureus.80436
26. Hazra Y, Rao A, Natarajan S, Srikrishna SB. Effect of herbal toothpaste on the colour stability, surface roughness, and microhardness of aesthetic restorative materials—an in vitro study. *BDJ Open.* 2024;10(1):95. doi:10.1038/s41405-024-00280-x

Ankyra Medical Journal
Original Article
DOI: 10.51271/ANKMJ-0065

# Evaluation of RPR, TPHA and ELISA test results used in the diagnosis of syphilis at a university hospital

Canberk Çınar*, Ahmet Tuğrul Yener, Demet Gür Vural, Yeliz Tanrıverdi Çaycı, Kemal Bilgin, Asuman Birinci

*Department of Medical Microbiology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkiye*

**Cite this article:** Çınar C, Yener AT, Gür Vural D, Tanrıverdi Çaycı Y, Bilgin K, Birinci A. Evaluation of RPR, TPHA and ELISA test results used in the diagnosis of syphilis at a university hospital. *Ank Med J.* 2026;5(2):46-49. doi: 10.51271/ANKMJ-0065

**Received**: 03/01/2026 ◆ **Accepted**: 28/01/2026 ◆ **Published**: 25/03/2026

## ABSTRACT

**Aims:** This study aimed to evaluate the laboratory results of treponemal and non-treponemal serological tests used in the diagnosis of syphilis and to investigate the diagnostic contributions of different testing algorithms.

**Methods:** Between April 2023 and June 2025, serum samples from 600 patients who underwent all three tests in the microbiology laboratory were retrospectively analyzed. The rapid plasma reagin (RPR), *Treponema pallidum* hemagglutination assay (TPHA) and enzyme-linked immunosorbent assay (ELISA) tests were performed according to the manufacturers' instructions, and the results were evaluated within both conventional and reverse algorithm frameworks.

**Results:** In 89.5% of cases (n=537), all three tests were negative, while at least one test was positive in 10.5% (n=63). ELISA showed the highest positivity rate, with 63 reactive samples. A total of 20 cases were positive across all three tests simultaneously. Samples positive only by ELISA were notably increased in the age group over 60 years. RPR positivity was lower compared to treponemal tests. Within the reverse algorithm, discrepancies between ELISA-positive and RPR-negative results were observed at a notable frequency.

**Conclusion:** Using treponemal tests as the first step in syphilis screening provides a more sensitive approach. Positive results should be confirmed with a second treponemal test, while non-treponemal tests are essential for assessing active infection and treatment monitoring. Higher ELISA positivity in older individuals suggests more false positives. The study underscores the need for laboratory–clinical collaboration and larger prospective studies.

**Keywords:** Syphilis, serologic tests, algorithms

## INTRODUCTION

Syphilis is a treatable infection caused by *Treponema pallidum*, transmitted sexually, and capable of multisystem involvement.[1] It is also referred to as "the French disease" due to its emergence among French soldiers.[2] Serological tests form the basis of laboratory diagnosis for syphilis. During infection, two types of antibodies are produced: treponemal and nontreponemal antibodies. Based on the antigens used, serological tests are classified as nontreponemal tests (rapid plasma reagin (RPR), venereal disease research laboratory (VDRL)) and treponemal tests (*Treponema pallidum* hemagglutination assay (TPHA), enzyme-linked immunosorbent assay (ELISA), and fluorescent treponemal antibody–absorption (FTA-ABS)).[3] The combined use of these tests provides a comprehensive approach for syphilis screening and diagnosis.[4] Diagnostic algorithms can be examined in three main groups: In the conventional algorithm, a nontreponemal test (RPR) is performed first, and positive samples are confirmed with a treponemal test. In the reverse algorithm, the first step is a treponemal test (ELISA/TPHA), and positive samples are confirmed with a nontreponemal test. The European Centre for Disease Prevention and Control (ECDC) recommendation involves using a positive treponemal screening test followed by a different second treponemal confirmation test.[5] Since treponemal tests can remain positive for life, they are not useful for treatment monitoring. In contrast, nontreponemal tests are more suitable for treatment follow-up due to the possibility of monitoring antibody titers.[6] The tests used in syphilis diagnosis have their own specific advantages and limitations; therefore, multiple tests are generally performed together. The validity of tests used in syphilis serology may vary depending on the applied treatment and disease stage. Thus, collaboration between clinicians and laboratories is crucial for accurate diagnosis. FTA-ABS test is a highly specific

**Corresponding Author:** Canberk Çınar, cinarcanberk57@gmail.com

confirmatory assay that detects antibodies against *Treponema pallidum* using fluorescent-labeled anti-human antibodies after absorption of nonspecific antibodies. It is widely accepted as the gold standard confirmatory test for syphilis and is particularly useful for confirming treponemal test reactivity in cases with discordant results.[7] This study aimed to evaluate the results of treponemal and nontreponemal tests used in syphilis diagnosis and to investigate the diagnostic contributions of different diagnostic algorithms.

## METHODS

This study was approved by the Ondokuz Mayıs University Ethics Committee for Clinical Researches (Date: 15.10.2025, Decision No: 2025/492). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This retrospective study was conducted using samples sent to the faculty of medicine serology/ELISA laboratory between April 2023 and June 2025. Ethical approval for the study was obtained under. Serum samples sent to the laboratory for preoperative screening, blood donor screening, premarital screening, and clinical suspicion were retrospectively evaluated. In our laboratory, the RPR (Monlab, Spain) is routinely used as the nontreponemal test, while TPHA (Monlab, Spain) and ELISA (Cobas 801, Syphilis TP; Roche Diagnostics, Germany) are employed as treponemal tests. Only cases in which all three tests were requested simultaneously were included in the study. A total of 600 patients were evaluated, and only the first serum sample from each patient was analyzed. The collected data were retrospectively analyzed according to both conventional and reverse algorithm approaches. In the conventional syphilis screening algorithm, a nontreponemal test such as the RPR test is used as the initial screening method, and reactive samples are subsequently confirmed with a treponemal test. In the reverse screening algorithm, serum samples are first screened using an automated treponemal test, such as ELISA. Reactive samples identified by ELISA are then tested with RPR to evaluate disease activity, treatment status, and to aid in determining the stage of infection.[8] Patient blood samples were initially centrifuged at 4000 rpm for 10 minutes, and subsequent testing was performed in accordance with the manufacturers' instructions.

**RPR test:** Fifty microliters (50 µL) of patient serum were placed in the circles on the card test, followed by the addition of 20 µL RPR reagent. The mixture was incubated on a rotator at 140 rpm for 8 minutes. The test was interpreted as positive in the presence of clumping (agglutination) and negative if a homogeneous appearance was observed.

**TPHA test:** Three microplate wells were prepared for each patient. In the first well, 190 µL TPHA diluent and 10 µL serum were added to prepare a 1/20 initial dilution, from which 25 µL was distributed into the other control and test wells. Subsequently, 75 µL of the control and test suspensions were added, and the plates were incubated at room temperature for 1 hour. Results were evaluated macroscopically: sediment formation at the bottom of the well was considered negative, whereas a homogeneous appearance indicated a positive reaction. Positivity at dilutions of 1/80 or higher was considered significant.

**ELISA test:** ELISA tests were performed on the Cobas 801 analyzer following the manufacturer's procedures. Results were interpreted according to the manufacturer's recommendations, with a COI ≥1.0 considered reactive (positive).

## RESULTS

The results of 600 patients tested at the Serology/ELISA Laboratory of Ondokuz Mayıs University Faculty of Medicine were retrospectively evaluated. Among the included cases, 279 (46.5%) were female and 321 (53.5%) were male. The mean age of the patients was 43.07 years. All three tests (RPR, TPHA, and ELISA) were negative in 537 samples (89.5%), while at least one test was positive in 63 samples (10.5%). The positive/negative distribution of the three tests is presented in Table 1. The comparative distribution of test results by year is shown in Table 2. Accordingly, the number of cases in which all three tests were simultaneously positive was 7 in 2023, 9 in 2024, and 4 in 2025. Notably, the number of samples in which ELISA was positive alone remained relatively high across all three years. Analysis of age distribution revealed that the majority of test requests were for patients aged 19–30 years. Comparative test results by age group are presented in Table 3. The cases in which all three tests were simultaneously positive were most frequently observed in the 19–40 age group, whereas cases in which ELISA was positive alone showed a marked increase in the 60 years and older age group.

**Table 1.** Number of patients testing positive by serological method

| Result | RPR | TPHA | ELISA |
|---|---|---|---|
| Pozitive | 21 | 39 | 63 |
| Negative | 579 | 561 | 537 |

RPR: Rapid plasma reagin, TPHA: *Treponema pallidum* hemagglutination assay, ELISA: Enzyme-linked immunosorbent assay

**Table 2.** Distribution of comparative test results by year

| Year | RPR+ TPHA+ ELISA+ | RPR- TPHA+ ELISA+ | RPR+ TPHA- ELISA- | RPR- TPHA+ ELISA- | RPR+ TPHA- ELISA+ | RPR- TPHA- ELISA+ | RPR+ TPHA+ ELISA- | RPR- TPHA- ELISA- |
|---|---|---|---|---|---|---|---|---|
| 2023 | 7 | 3 | 0 | 0 | 0 | 6 | 0 | 240 |
| 2024 | 9 | 16 | 0 | 0 | 1 | 15 | 0 | 267 |
| 2025 | 4 | 0 | 0 | 0 | 0 | 2 | 0 | 30 |
| Number of patients | 20 | 19 | 0 | 0 | 1 | 23 | 0 | 537 |

RPR: Rapid plasma reagin, TPHA: *Treponema pallidum* hemagglutination assay, ELISA: Enzyme-linked immunosorbent assay

**Table 3.** Distribution of comparative test results by age

| Age | RPR+ TPHA+ ELISA+ | RPR- TPHA+ ELISA+ | RPR+ TPHA- ELISA- | RPR- TPHA+ ELISA- | RPR+ TPHA- ELISA+ | RPR- TPHA- ELISA+ | RPR+ TPHA+ ELISA- | RPR- TPHA- ELISA- |
|---|---|---|---|---|---|---|---|---|
| 0-18 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 32 |
| 19-30 | 5 | 2 | 0 | 0 | 0 | 2 | 0 | 132 |
| 31-40 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 105 |
| 41-50 | 4 | 9 | 0 | 0 | 0 | 2 | 0 | 88 |
| 51-60 | 1 | 3 | 0 | 0 | 1 | 2 | 0 | 81 |
| 60< | 4 | 3 | 0 | 0 | 0 | 15 | 0 | 99 |
| Number of patients | 20 | 19 | 0 | 0 | 1 | 23 | 0 | 537 |

RPR: Rapid plasma reagin, TPHA: *Treponema pallidum* hemagglutination assay, ELISA: Enzyme-linked immunosorbent assay

## DISCUSSION

An increase in the incidence of syphilis infections has been reported in many countries worldwide.[9] Syphilis, which affects both men and women, is frequently observed in sexually active age groups, individuals with suspected sexual exposure, homosexual men, and populations with lower socioeconomic status. Major transmission routes include sexual contact, transplacental transfer, and blood transfusion.[10] The reverse algorithm offers several advantages, including early detection of cases and the ability to avoid missing past syphilis infections that may not be detected by nontreponemal tests. However, reverse algorithm results can cause confusion and concern among patients and laboratory personnel, particularly when ELISA and RPR results are discordant (e.g., ELISA reactive, RPR nonreactive). Such results typically reflect successfully treated past syphilis cases but may also appear in non-syphilis conditions (e.g., false-reactive ELISA) or in early, late, or latent syphilis where RPR sensitivity is low.[11] In a study evaluating the potential impact of the reverse algorithm on syphilis diagnosis, approximately 140.000 serum samples screened initially with ELISA were analyzed. Samples were collected from patients residing in areas of low or high syphilis prevalence. About 3.4% (4,834) of samples tested by ELISA were reactive, and 56.7% (2,743) of these were nonreactive by RPR. Discordant samples were further tested with FTA-ABS, and 31.6% (833) were found to be nonreactive, suggesting false-positive ELISA results.[12] Another study comparing the screening superiority of ELISA versus RPR demonstrated several advantages of ELISA over conventional flocculation screening tests. Unlike RPR, ELISA provides more objective results and is not affected by the prozone phenomenon or the stage of syphilis infection. However, ELISA does not achieve 100% sensitivity and specificity, cross-reactivity can occur, and combined testing is recommended.[13] In a related study examining 1000 patient samples, it was emphasized that the conventional algorithm should ideally not be used for screening and that treponemal and nontreponemal tests should be applied together, with discordant results, if possible, evaluated with a second treponemal test. The study also highlighted that treponemal tests used in the reverse algorithm may produce false-positive results, especially in older patients, and that ELISA reactivities with COI <20 U were mostly negative when checked with FTA-ABS, yet treponemal tests should still be selected as the first-line test.[14] It is well known that nontreponemal test performance depends on the stage of infection. Sensitivity can decrease to 60–70% in the primary stage, potentially leading to missed asymptomatic cases in early infection.[15] In our study, the lower RPR positivity compared to treponemal tests aligns with this information, suggesting that using nontreponemal tests alone in screening may result in underdiagnosis and that treponemal-based screening is more appropriate. Another advantage of the reverse algorithm is the potential for automation. New-generation automated treponemal tests, such as Alinity, Architect, and Elecsys, have been reported to be successful in blood donor screening, offering low false-positive rates and high workflow efficiency.[16]

### Limitations

However, limitations of the reverse algorithm should also be considered. Despite the high sensitivity of treponemal screening tests, false positivity has been reported in older adults, patients with autoimmune diseases, pregnancy, and certain viral infections.[7] In our study, higher ELISA positivity in patients aged 60 and older may support the notion of age-related reduced specificity. The 20 patients who were positive across all three tests (RPR, TPHA, and ELISA) were observed in all age groups, with the youngest group being 0–18 years and the oldest group being over 60 years. Additionally, a recent evaluation by Ortiz et al.[17] emphasized that the reverse algorithm can create confusion for clinicians, particularly in ELISA-positive/RPR-negative samples, highlighting the necessity of reflex confirmatory testing. In the reverse algorithm, discordant results (treponemal test positive and nontreponemal test negative) may be observed. In such cases, a reflex confirmatory test is recommended, meaning that the laboratory automatically performs a second, different treponemal assay (e.g., TPPA or FTA-ABS) on the same sample to confirm true infection and rule out false-positive screening results without requiring a new sample or separate physician request.[17] A major limitation of our study is the lack of additional clinical data accompanying laboratory results. Interpretation of treponemal test positivity was challenging without information on treatment history, previous test results, and clinical findings. Moreover, the inability to perform FTA-ABS, the gold standard confirmatory test, limited the evaluation of potential false-positive or false-negative results.

## CONCLUSION

Overall, our study reinforces that treponemal tests are suitable as the first step in syphilis screening, that treponemal test positivity should always be confirmed with a different

second treponemal method, and that nontreponemal tests play an indispensable role in assessing active infection and monitoring treatment. Larger prospective studies evaluating algorithm performance across different age groups and clinical risk levels are warranted. A fundamental challenge in interpreting syphilis serology is that treponemal tests remain positive for life, meaning they are not sufficient alone to distinguish between past and active infection. The increasingly adopted reverse algorithm provides significant advantages for early detection of asymptomatic and latent cases. In our study, the notable frequency of ELISA-positive/RPR-negative samples aligns with the sensitivity advantage offered by the reverse algorithm, which is also strongly supported by recent literature.

## ETHICAL DECLARATIONS

### Ethics Committee Approval

This study was approved by the Ondokuz Mayıs University Ethics Committee for Clinical Researches (Date: 15.10.2025, Decision No: 2025/492).

### Informed Consent

As this was a retrospective study, formal written informed consent was not required and was therefore not obtained.

### Peer Review Process

This manuscript was subject to external peer review.

### Conflict of Interest

The authors declare no conflicts of interest related to this study.

### Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

### Author Contributions

Concept: CÇ, ATY; Design: CÇ, YTÇ, AB; Control: DGV, KB; Resources: AB, YTÇ; Materials: ATY, CÇ, YTÇ; Data Collection and/or Processing: AB, KB, DGV; Analysis and/or Interpretation: AB, KB, YTÇ; Literature Review: CÇ, ATY; Writing the Article: CÇ, KB, ATY; Critical Review: CÇ, YTÇ, DGV, ATY, KB, AB.

## REFERENCES

1. Peeling RW, Mabey D, Chen XS, Garcia PJ. Syphilis. *Lancet*. 2023; 402(10398):336-346. doi:10.1016/S0140-6736(22)02348-0
2. Forrestel AK, Kovarik CL, Katz KA. Sexually acquired syphilis: laboratory diagnosis, management and prevention. *J Am Acad Dermatol*. 2020;82(1):17-28. doi:10.1016/j.jaad.2019.02.074
3. Şamlıoğlu P. Evaluation of one-year rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination assay (TPHA) results in a tertiary public hospital. *Clin Med Sci*. 2018;6(2):9-11. doi:10.5222/TMCD.2018.0
4. Basgalupp S, Dornelles T, Pedrotti L, et al. Diagnostic properties of different serological methods for syphilis testing in Brazil. *Diagnostics (Basel)*. 2025;15(12):1448. doi:10.3390/diagnostics15121448
5. Simčič S, Potočnik M. Serological diagnosis of syphilis: a comparison of different diagnostic methods. *Acta Dermatovenerol Alp Pannonica Adriat*. 2015;24(2):17-20. doi:10.15570/actaapa.2015.6
6. Vural DG, Ateş E, Çaycı YT, Bilgin K, Birinci A. Evaluation of five-year rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination assay (TPHA) results in a university hospital. *Value Health Sci*. 2024; 14(2):198-203. doi:10.5222/ValueHealthSci.2024.198
7. Karaca Y, Çöplü N, Gözalan A, Öncül Ö, Akın L, Esen B. Establishment of an algorithm for serological testing of syphilis identification. *Mikrobiyol Bul*. 2010;44(1):35-45. doi:10.5578/mb.8891
8. Binnicker MJ, Jespersen DJ, Rollins LO. Direct comparison of the traditional and reverse syphilis screening algorithms in a population with a low prevalence of syphilis. *J Clin Microbiol*. 2012;50(1):148-150. doi:10.1128/JCM.05636-11
9. Sönmez C. Evaluation of RPR and TPHA test results and diagnostic algorithms used in syphilis diagnosis. *Turk J Med Sci*. 2018;48(1):72-77. doi:10.3906/sag-1706-100
10. Ziver T, Yüksel P, Güngördü Z, İzmirli S. Evaluation of rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination assay (TPHA) test results used in the diagnosis of syphilis infections between 2005 and 2010. *Turk J Hyg Exp Biol*. 2011;68(1):1-7. doi:10.5505/TurkHijyen.2011.88700
11. Seña AC, White BL, Sparling PF. Novel *Treponema pallidum* serologic tests: a paradigm shift in syphilis screening for the 21st century. *Clin Infect Dis*. 2010;51(6):700-708. doi:10.1086/655832
12. Radolf JD, Bolan G, Park IU, et al. Discordant results from reverse sequence syphilis screening—five laboratories, United States, 2006-2010. *MMWR Morb Mortal Wkly Rep*. 2011;60(5):133-157.
13. Negash M, Wondmagegn T, Geremew D. Comparison of RPR and ELISA with TPHA for the diagnosis of syphilis: implication for updating syphilis point-of-care tests in Ethiopia. *J Immunol Res*. 2018; 2018:2978419. doi:10.1155/2018/2978419
14. Nah EH, Cho S, Kim S, Cho HI, Chai JY. Comparison of traditional and reverse syphilis screening algorithms in medical health checkups. *Ann Lab Med*. 2017;37(6):511-515. doi:10.3343/alm.2017.37.6.511
15. Clement ME, Okeke NL, Hicks CB. Treatment of syphilis: a systematic review. *JAMA*. 2014;312(18):1905-1917. doi:10.1001/jama.2014.13259
16. Cheng A, Das A, Styles CE, Naing Z, Rawlinson WD, Gosbell IB. Improved efficiency using sequential automated immunoassays for syphilis screening in blood donors. *J Clin Microbiol*. 2024;62(8): e0047624. doi:10.1128/jcm.00476-24
17. Ortiz DA, Shukla MR, Loeffelholz MJ. The traditional or reverse algorithm for diagnosis of syphilis: pros and cons. *Clin Infect Dis*. 2020; 71(Suppl 1):S43-S51. doi:10.1093/cid/ciaa307

Ankyra Medical Journal
Review
DOI: 10.51271/ANKMJ-0066

# Tumor lysis syndrome

Beyza Hilal Balcı*[1], Selim Yalçın[2]

[1]Department of Internal Medicine, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkiye
[2]Division of Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkiye

Cite this article: Balcı BH, Yalçın S. Tumor lysis syndrome. *Ank Med J.* 2026;5(2):50-55. doi: 10.51271/ANKMJ-0066

Received: 31/12/2025 ◆ Accepted: 25/01/2026 ◆ Published: 25/03/2026

## ABSTRACT

Tumor lysis syndrome (TLS) is defined as the release of intracellular metabolites into the bloodstream, resulting from the breakdown of tumor cells due to various causes encountered in clinical practice. It has been observed that TLS, which occurs in patients with existing malignancy, further worsens the prognosis in the already poor condition and increases the risk of mortality in patients. Therefore, the diagnosis and follow-up of TLS by relevant clinicians is of great importance. This review examines overlooked aspects of TLS, including its etiology, pathophysiology, necessary investigations, diagnosis, treatment algorithms, and current clinical approach.

**Keywords:** Oncology, oncological emergency, tumor lysis syndrome

## INTRODUCTION

Tumor lysis syndrome (TLS) is a condition characterized by the release of intracellular metabolites into the bloodstream, resulting from the breakdown of tumor cells. Its characteristic findings include hyperuricemia, hyperphosphatemia, hyperkalemia, and hypocalcemia. These electrolyte disturbances result in cardiac and renal effects, which can be life-threatening.[1]

TLS, which occurs in various malignancies, is associated with a poor prognosis because it occurs in patients who are currently undergoing chemotherapy and are in poor general condition. In addition to increasing mortality and morbidity in patients, it also creates extra costs for hospitals.[2]

## ETIOLOGY AND RISK FACTORS

Furthermore, it has been determined that some solid tumors can also cause TLS. Among these, hepatoblastoma and neuroblastoma are more commonly seen. Although rare, cases of TLS occurring spontaneously before the start of chemotherapy have also been described in the literature.[3]

Based on studies, tumors that cause TLS have been classified by risk and summarized in Table 1.[4]

Although TLS is frequently associated with hematologic malignancies, it has also been observed to develop as a result of certain solid tumors. It primarily occurs in association with hepatoblastoma and neuroblastoma, while other related tumors are summarized in Table 2.[5]

**Table 2.** Solid tumors associated with tumor lysis syndrome

| Solid tumors associated with tumor lysis syndrome |
|---|
| • Germ cell tumors<br>• Neuro- and medulla blastomas<br>• Small cell carcinoma and other lung tumors<br>• Breast, ovarian, and vulvar neoplasms<br>• Hepatoblastoma and hepatocellular carcinoma<br>• Colorectal and gastric carcinoma<br>• Melanoma<br>• Sarcoma |

**Table 1.** Classification of tumors according to their risk of developing tumor lysis syndrome

| High-risk tumors | Intermediate-risk tumors | Low-risk tumors |
|---|---|---|
| Acute lymphocytic leukemia (5.2% to 23%)<br>Acute myeloid leukemia with a WBC count greater than 75,000 (18%)<br>B-cell acute lymphoblastic leukemia (26.4%)<br>Burkitt lymphoma (14.9%) | Acute myeloid leukemia with a WBC count between 25,000 and 50,000 (6%)<br>Diffuse large B-cell lymphoma (6%) | Acute myeloid leukemia with a WBC count less than 25,000 (1%)<br>Chronic lymphocytic leukemia (0.33%)<br>Chronic myelogenous leukemia (case reports)<br>A solid tumor (case reports) |

WBC: White blood cell

**Corresponding Author:** Beyza Hilal Balcı, beyzaharpaci@gmail.com

In recent clinical studies on TLS, it has been determined that the chemotherapy regimen and biological agents administered also pose a risk for TLS. Chemotherapeutic and biological agents that are more frequently associated with TLS are summarized in Table 3.[4,6,7]

**Table 3.** Chemotherapeutic agents and biological agents that more frequently cause tumor lysis syndrome

| | |
|---|---|
| Thalidomide | Venetoclax (BCL2 inhibitor) |
| Bortezomib | Obinutuzumab, rituximab (anti-CD20 monoclonal antibody) |
| Hydroxyurea | Nivolumab, pembrolizumab (anti-PD-1 monoclonal antibody) |
| Paclitaxel | Dinaciclib, alvocidib (cyclin-dependent kinase inhibitor) |
| Fludarabine | |
| Etoposide | |
| Zoledronic acid | |

Studies on risk factors associated with the development of TLS have revealed that the increased risk is not solely related to tumor type but also to certain patient-specific factors. Accordingly, male gender, advanced age, underlying chronic kidney disease, and the presence of accompanying conditions have been found to increase the risk. The disease is generally classified into three risk categories. Accordingly, it is classified as high risk (TLS develops in more than 5% of patients), medium risk (TLS develops in 1-5% of patients), and low risk (TLS develops in less than 1% of patients). Risk factors associated with TLS are summarized in Table 4.[8-10]

**Table 4.** Tumor type and patient-related risk factors associated with tumor lysis syndrome

| Tumor risk factors | Patient-related risk factors |
|---|---|
| • Type of tumor<br>• Tumor volume (tumors >10 cm)<br>• Metastatic disease<br>• Tumor growth rate (LDH>2 times normal value)<br>• Leukocytosis (>25,000/mm$^3$)<br>• Sensitivity to chemotherapy (germ cell tumors, small cell lung cancer, etc) | • Male gender<br>• Age >65 years<br>• Pretreatment serum creatinine >1.4 mg/dl<br>• Renal obstruction<br>• Pretreatment serum uric acid >7.5 mg/dl<br>• Associated conditions (hypotension, hypovolemia, nephrotoxic drugs, chronic kidney disease) |

Spontaneous TLS is the spontaneous development of aggressive tumors due to an excessively rapid cell turnover, without any chemotherapy or radiotherapy support. This clinical picture is frequently observed in malignancies such as Burkitt lymphoma and acute lymphoblastic leukemia (ALL), and can sometimes appear as the first clinical sign of an as yet undiagnosed cancer case.[11] Statistical data show that this condition develops spontaneously in approximately 28% to 55.6% of childhood ALL cases.[12] Unlike treatment-induced TLS, hyperphosphatemia is generally milder in spontaneous cases because the viable tumor cells can metabolize the released phosphate for new cell production. However, since nucleic acid accumulation strains the body's detoxification capacity, uric acid concentrations can climb to risky levels. This condition, previously thought to be rare in solid tumors, is now reported more frequently thanks to improved monitoring methods and intensive treatment protocols. According to data, approximately 24% of TLS cases in solid tumors occur spontaneously. On the other hand, not only systemic drug therapies but also local interventions applied to liver tumors, such as TACE or radiofrequency ablation (RFA), carry a risk of triggering this syndrome.[13,14]

In modern oncology practice, TLS cases are being encountered more frequently as a result of the superior success of targeted and biological drugs in destroying cancer cells. Chronic lymphocytic leukemia (CLL) and multiple myeloma, which were previously considered low-risk, are now classified as high-risk diseases with the introduction of innovative methods such as venetoclax, obinutuzumab, rituximab, and CAR-T cellular therapies. Ironically, the rapid and intense destructive effect of these drugs on malignant cells can trigger serious metabolic disorders that endanger the patient's life.[15,16] Therefore, when assessing risk in current treatment protocols, not only the type of cancer should be focused on; the potency of the chosen therapeutic agent must also be considered as a vital criterion.

## EPIDEMIOLOGY

Although the incidence of TLS has not been definitively established, clinical data have provided insight into which tumor types may be associated with secondary TLS. TLS is most commonly seen in hematological malignancies. The highest risk is observed in Burkitt lymphoma, ALL, and high-grade non-Hodgkin lymphoma (NHL). According to US data, 30% of patients discharged with a diagnosis of TLS are from NHL, 19% from acute myeloid leukemia (AML), and 13% from ALL. While this rate is not very high in solid tumors, small cell lung cancer, breast cancer, germ cell tumors, and hepatocellular carcinoma are the most common. Interestingly, TLS occurs spontaneously before treatment in 24% of these cases. Incidence data by tumor type are indicated in parentheses in Table 1.[4,17,18]

Pediatric patients are at higher risk because they are more affected by proliferative diseases. Burkitt lymphoma has the highest risk, at 30%, as in adults. In T-cell ALL and hyperviscosity cases, the risk of TLS increases to 20%. TLS is relatively rare in AML because the proliferation rate is lower.[11] Although TLS is rare in solid tumors, it has been reported in chemosensitive and bulky tumors such as hepatoblastoma, neuroblastoma, and germ cell tumors.[18] The risk is particularly significant in stage IV neuroblastoma.[11]

## PATHOPHYSIOLOGY

The pathophysiology of TLS involves a complex combination of biochemical pathways. TLS is primarily based on an increase in products metabolized to uric acid and electrolyte imbalance. The DNA chain is composed of molecules called nucleotides. Nucleotides consist of a phosphate group, a sugar group, and a nitrogen base. The nitrogen bases are adenine, thymine, guanine, or cytosine. Adenine and guanine are called purines, while cytosine and thymine are called pyrimidines.[19] Purines are among the primary products metabolized into uric acid. Intermediate products are produced as a result of purine metabolism. These products can be listed as hypoxanthine and xanthine. Adenine is metabolized into hypoxanthine, while guanine is metabolized into xanthine. Xanthine is then converted into uric acid through a reaction catalyzed by xanthine oxidase. Under normal conditions, uric acid is excreted via the kidneys. Although many mammalian groups have the enzyme urate oxidase, which converts uric acid into its more soluble form, allantoin, humans do not. Figure 1 summarizes purine metabolism.[20]

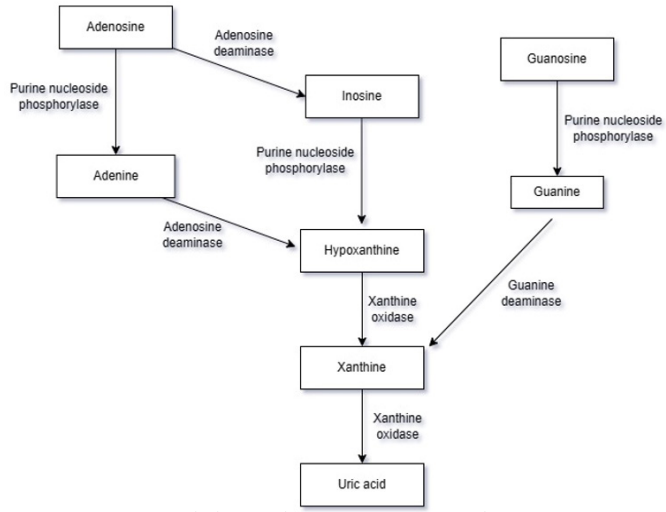


**Figure 1.** Purine metabolism and conversion to uric acid

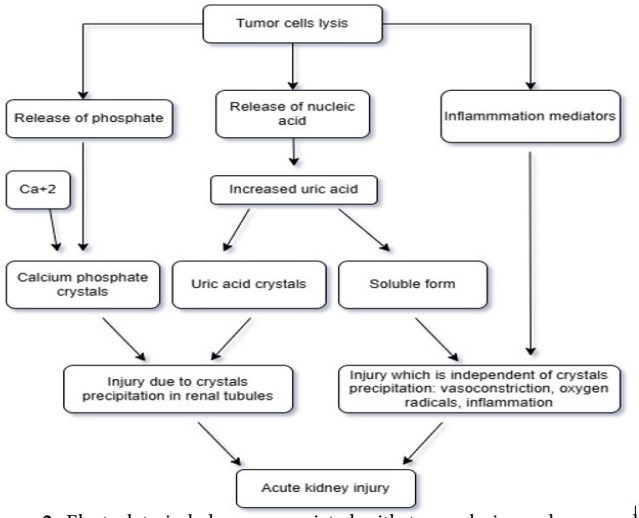


**Figure 2.** Electrolyte imbalances associated with tumor lysis syndrome and their relationship with kidney damage

Uric acid, which is produced as a result of purine metabolism, is normally excreted through the kidneys. However, in TLS, the release of large amounts of uric acid causes it to crystallize in the tubules and can lead to kidney damage. It has also been determined that uric acid crystals cause kidney damage by inducing the release of free oxygen radicals and stimulating vasoconstriction and inflammation.[21]

Many electrolytes are also released as a result of tumor cell destruction. The intracellular concentration of potassium is approximately 120-130 mEq/L. Potassium released from tumor cell breakdown is largely compensated for by the liver and skeletal muscle. However, in cases of excessive release, the compensation threshold is exceeded, serum potassium levels increase, and cardiac arrhythmias, particularly in the setting of hypokalemia, may occur.[22]

Another electrolyte imbalance associated with TLS is hyperphosphatemia. Intracellular phosphate enters the bloodstream as a result of cell breakdown. Under normal conditions, phosphate, which is excreted by the kidneys, precipitates with calcium and accumulates in the renal tubules, causing kidney damage.[23]

The precipitation of phosphate with calcium also lowers serum calcium levels. This is much more important than the clinical consequences of hyperphosphatemia. Due to the importance of calcium in cardiac rhythm, life-threatening situations may occur. In these patients, arrhythmias, tetany, and seizures may develop. Electrolyte imbalances and their effects associated with TLS are summarized in **Figure 2**.[24,25]

## CLINICAL FINDINGS AND DIAGNOSTIC APPROACH

TLS does not present specific clinical findings. Clinical findings are mostly due to electrolyte and metabolite abnormalities caused by the syndrome. Therefore, patients may develop fatigue, weakness, and ECG abnormalities due to hyperkalemia. Due to hypocalcemia, spasms, tetany, positive Chvostek and Trousseau signs, and seizures may be observed. Due to hyperuricemia and obstructive uropathy, symptoms such as weakness, fatigue, irritability, nausea, vomiting, and itching may be observed.[26]

The diagnosis of TLS is based on the criteria developed by Cairo and Bishop. Although this classification is widely used, it has limitations in some areas. The most important disadvantage of the classification is that it is based on the development of the syndrome after patients have received chemotherapy. However, it is known that TLS can also develop in patients who have not yet started chemotherapy. Another disadvantage is that a serum creatinine level exceeding 1.5 times the normal level is one of the key criteria in the clinical diagnosis of TLS. This is because creatinine is considered a poor biomarker for detecting acute kidney injury, as it is heavily influenced by factors such as age, muscle mass, and hydration status. Furthermore, current criteria struggle to differentiate newly developed acute injury in chronic kidney disease patients who already have elevated baseline creatinine levels, as these patients may have creatinine values 1.5 times above the normal range even before developing acute kidney injury. On the other hand, impaired kidney function may not always be due to clinical TLS; conditions such as dehydration, use of nephrotoxic drugs (vancomycin, contrast agents, etc.), or direct infiltration of the kidneys by the tumor can mimic the criteria of TLS.[27]

The second disadvantage is the use of a 1.5-fold limit for serum creatinine levels. Conditions such as elevated baseline creatinine levels in patients with chronic kidney disease raise questions about the standardization of this criterion. Nevertheless, the Cairo and Bishop classification is the most clinically accepted and widely used classification and is summarized in **Table 5**.[28]

A staging system is used to evaluate treatment approaches for TLS. Although the staging system developed by Cairo and Bishop has been criticized by some authors for its definition of elevated creatinine, it is currently used clinically. The Cairo-Bishop TLS staging system is summarized in **Table 6**.[29] Although the Cairo-Bishop classification is a generally accepted classification, some of its limitations have been criticized in studies. The Cairo-Bishop definition limits TLS to the period between 3 days before and 7 days after the start of chemotherapy. However, in clinical practice, TLS can develop spontaneously before any treatment begins and this may initially be overlooked.[18] In addition, while a 25%

**Table 5.** Cairo and Bishop classification used in tumor lysis syndrome

| Cairo-Bishop definition of tumor lysis syndrome (TLS) | | | |
|---|---|---|---|
| Laboratory TLS<br>Defined by the modification of at least 2 parameters within 24 hours. | Uric acid ≥8 mg/dl<br>Potassium ≥6 mg/dl<br>Phosphate ≥4.5 mg/dl | or 25% increase | The condition typically occurs within 3 to 7 days after chemotherapy initiation |
| | Calcium ≤7 mg/dl | or 25% decrease | |
| Clinical TLS<br>Defined as laboratory TLS plus one organ dysfunction or death. | Renal dysfunction (creatinine 1.5× normal values)<br>Cardiac involvement (arrhythmias)<br>Neurological involvement (seizures, tetany)<br>Death | | |

**Table 6.** Cairo and Bishop staging system used in tumor lysis syndrome

| | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Creatinine | ≤1.5xULN | >1.5xULN | 1.5-3xULN | 3-6xULN | >6xULN | Death |
| Arrhythmias | None | Intervention not indicated | Intervention indicated | Symptomatic, incompletely controlled | Life threatening | Death |
| Seizures | None | - | Single, easily controlled | Repeated, altered consciousness | Prolonged refractory | Death |

ULN: Upper limit of normal

change from the baseline value is considered sufficient in laboratory TLS criteria, some clinicians argue that this 25% variation is not clinically significant and may increase the false positive rate, especially if the absolute values are within normal limits.[11,30] While the original criteria did not require the simultaneous occurrence of metabolic abnormalities, some researchers, such as Howard et al.,[29] have stated that individual abnormalities developing at different times may be unrelated to TLS, and therefore, the diagnosis should be based on the presence of at least two abnormalities within the same 24-hour period. Some experts argue that the "stage 0" definition in the Cairo-Bishop system is unnecessary and that more specific systems are needed for grading clinical complications (arrhythmia, seizures, etc.).[31] However, the shortcomings and difficulties related to the evaluation of renal function have also been mentioned above.

While the ASCO (2008), NCCN, and 2010 International Expert Panel Consensus (generally used as a basis by ESMO) guidelines on the management of TLS show significant similarities on a scientific basis, they have some shortcomings and differences in terms of risk assessment models and application details. Most guidelines accept the 2004 Cairo-Bishop criteria as the standard for diagnosis.[32] The biggest difference between the guidelines is on risk categorization and the effect of renal function on this risk. The 2010 International Consensus offers the most comprehensive and systematic model, classifying patients into "low, intermediate, high" risk groups. In contrast, the NCCN generally presents TLS risk by integrating it into disease-specific guidelines such as leukemia or lymphoma. The 2010 Consensus uses existing renal failure or dehydration as a "risk modulator"; that is, a low or intermediate-risk patient is automatically moved to a higher risk group if renal function is impaired.[32,33] ASCO (2008), however, focuses more on adult data and does not address renal function variables in the pediatric group in the same depth.[28]

## TREATMENT APPROACH

First and foremost, prophylaxis should be prioritized in patients at high risk of developing TLS. The goal of adequate treatment is to prevent acute kidney injury and electrolyte imbalances that pose a major life-threatening risk. Therefore, in patients who have started cytoreductive therapy, renal function tests, serum electrolytes, and uric acid should be monitored at specific intervals based on risk category. The monitoring intervals for each group are summarized in Table 7.[29]

**Table 7.** Frequency of monitoring tests according to risk stages in tumor lysis syndrome

| | |
|---|---|
| Patients at high risk | every 4 to 6 hours after antitumor therapy initiation |
| Patients at intermediate risk | every 8 to 12 hours after antitumor therapy initiation |
| Patients at low risk | daily |

Intravenous hydration and ensuring adequate urine output are considered fundamental steps in the management of TLS. Guidelines recommend aggressive hydration, particularly in the moderate- and high-risk groups. As a result, glomerular filtration and urine output increase, reducing the likelihood of electrolyte and uric acid crystal precipitation in the renal tubules. Although there is no clear preference for intravenous fluid, Ringer's lactate should not be used due to its high potassium content.[34]

One of the key monitoring parameters following hydration is urine output. The recommended urine output is 80-100 ml/m² per hour. Diuretics may be preferred to achieve this output. Loop diuretics are the first-line diuretics. The reason for preferring loop diuretics is that they significantly increase potassium excretion.[35]

Hyperkalemia is a life-threatening electrolyte imbalance in TLS and requires acute intervention. In these patients, dietary potassium must be reduced, intravenous glucose-insulin solutions should be administered in cases of severe elevation, and loop diuretics should be preferred when necessary. Intravenous calcium gluconate should be administered to achieve cardiac stabilization in cases where cardiac risk is present.[29]

Hypocalcemia and hyperphosphatemia are also conditions that require prompt correction. Oral phosphate binders should be preferred for hyperphosphatemia when necessary,

and hypocalcemia should be managed with replacement therapy. Long-term phosphate stabilization has been shown to be beneficial in preventing the need for renal replacement therapy.[36]

In recent years, urinary alkalization has been actively used to increase the solubility of uric acid in urine. Sodium bicarbonate has been administered for urinary alkalization, aiming to reduce tubular precipitation of uric acid. However, recent studies have not shown that urine alkalization prevents uric acid precipitation; on the contrary, they have shown that it can cause calcium phosphate deposits in various organs. Therefore, the use of sodium bicarbonate for urine alkalization is not recommended today.[37]

Other methods for lowering uric acid involve directly suppressing uric acid synthesis and increasing uric acid breakdown. Currently, three hypouricemic agents are used with these methods. Allopurinol and febuxostat inhibit uric acid production, while rasburicase increases its breakdown. Hypouricemic agents and their mechanisms of action are summarized in **Table 8**.[32]

**Table 8.** Hypouricemic agents and mechanisms of action

| | |
|---|---|
| Allopurinol | Xanthine oxidase inhibitors |
| Febuxostat | |
| Rasburicase | Recombinant urate oxidase |

Allopurinol inhibits the formation of new uric acid by inhibiting xanthine oxidase. Because it affects the formation of new uric acid, it is administered prophylactically 24-48 hours before treatment. Studies have shown that it has no effect on pre-existing serum uric acid levels.[38] Its low cost and oral administration are its main advantages. However, as a result of inhibiting uric acid formation, serum levels of hypoxanthine and xanthine, which are precursors of uric acid, increase significantly. Therefore, it has been observed that it increases the risk of causing acute kidney injury by accumulating in the tubular lumen.[39]

Furthermore, allopurinol has been found to interact with various chemotherapeutic agents, particularly methotrexate.[40] In addition, the need for dose adjustment in renal function is another disadvantage.[41] Febuxostat is an agent that, like allopurinol, acts by inhibiting xanthine oxidase and is administered orally.

Febuxostat has been found to be superior to allopurinol in many ways. These include not requiring dose adjustment in mild to moderate renal impairment,[42] fewer drug interactions,[43] and greater uric acid-lowering effects.[44,45] On the other hand, its high cost is a factor that significantly limits its use.

Rasburicase is a recombinant form of urate oxidase that converts uric acid into allantoin, which is more soluble in water, has a lower toxicity risk, and is more easily excreted in urine. It has been shown to rapidly and effectively resolve TLS.

Rasburicase is often preferred in high-risk patients with TLS or in patient groups for whom xanthine oxidase inhibitors are contraindicated.[46] Renal replacement therapy should be considered without delay in patients who do not respond adequately to hypouricemic agents and who develop acute kidney injury and resistant electrolyte disturbances as a result.

While clinical guidelines align on the use of rasburicase for high-risk patients, the selection between allopurinol and rasburicase for moderate-risk individuals remains largely at the clinician's discretion due to a shortage of strong evidence-based findings.[31] Although most protocols advocate for weight-dependent dosing (0.15 - 0.2 mg/kg), recent studies suggest that fixed doses of 3 mg or 6 mg may be effective in adults, even though this methodology has not yet been integrated into all primary guidelines as a standard procedure.[15]

Currently preferred renal replacement therapies include daily hemodialysis, continuous venovenous hemofiltration, intermittent hemodialysis, and continuous hemofiltration. Renal replacement therapy should be continued until urine output and electrolytes return to normal.[47]

## CONCLUSION

All these treatment methods should correct the kidney damage and electrolyte imbalances caused by TLS. Otherwise, it poses a life-threatening risk and leads to increased mortality.

## ETHICAL DECLARATIONS

### Peer Review Process

This review was externally peer-reviewed.

### Conflict of Interest

The authors declare no conflicts of interest.

### Financial Disclosure

No financial support was received for the preparation or publication of this article.

### Author Contributions

Concept: BHB, SY; Design: BHB, SY; Control: BHB, SY; Data Collection and/or Processing: BHB, SY; Analysis and/or Interpretation: BHB, SY; Literature Review: BHB, SY; Article Writing: BHB, SY; Critical Review: All Authors.

## REFERENCES

1. Wierda WG, Byrd JC, O'Brien S, et al. Tumour debulking and reduction in predicted risk of tumour lysis syndrome with single-agent ibrutinib in patients with chronic lymphocytic leukaemia. *Br J Haematol.* 2019; 186(1):184-188. doi:10.1111/bjh.15791
2. McGhee-Jez A, Batra V, Sunder T, Rizk S. Spontaneous tumor lysis syndrome as presenting sign of metastatic prostate cancer. *Cureus.* 2018;10(12):e3706. doi:10.7759/cureus.3706
3. Williams SM, Killeen AA. Tumor lysis syndrome. *Arch Pathol Lab Med.* 2019;143(3):386-393. doi:10.5858/arpa.2017-0278-RS
4. Adeyinka A, Bashir K. Tumor lysis syndrome. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.
5. Baeksgaard L, Sørensen JB. Acute tumor lysis syndrome in solid tumors--a case report and review of the literature. *Cancer Chemother Pharmacol.* 2003;51(3):187-192. doi:10.1007/s00280-002-0556-x
6. Miao L, Zhang Z, Ren Z, Li Y. Reactions related to CAR-T cell therapy. *Front Immunol.* 2021;12:663201. doi:10.3389/fimmu.2021.663201
7. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med.* 2018;378(5):439-448. doi:10.1056/NEJMoa1709866

8. Wössmann W, Schrappe M, Meyer U, Zimmermann M, Reiter A. Incidence of tumor lysis syndrome in children with advanced stage Burkitt's lymphoma/leukemia before and after introduction of prophylactic use of urate oxidase. *Ann Hematol*. 2003;82(3):160-165. doi:10.1007/s00277-003-0608-2
9. Mato AR, Riccio BE, Qin L, et al. A predictive model for the detection of tumor lysis syndrome during AML induction therapy. *Leuk Lymphoma*. 2006;47(5):877-883. doi:10.1080/10428190500404662
10. Truong TH, Beyene J, Hitzler J, et al. Features at presentation predict children with acute lymphoblastic leukemia at low risk for tumor lysis syndrome. *Cancer*. 2007;110(8):1832-1839. doi:10.1002/cncr.22990
11. Cheung WL, Hon KL, Fung CM, Leung AK. Tumor lysis syndrome in childhood malignancies. *Drugs Context*. 2020;9:2019-8-2. doi:10.7573/dic.2019-8-2
12. Andarsini MR, Hartono A, Cahyadi A, Larasati MCS, Asmara APA. Risk factors of tumor lysis syndrome in childhood acute lymphoblastic leukemia. *Univ Med*. 2024;43(3):265-271. doi:10.18051/UnivMed.2024.v43.265-271
13. Bercovitz RS, Greffe BS, Hunger SP. Acute tumor lysis syndrome in a 7-month-old with hepatoblastoma. *Curr Opin Pediatr*. 2010;22(1):113-116. doi:10.1097/MOP.0b013e32833499e5
14. Joshita S, Yoshizawa K, Sano K, et al. A patient with advanced hepatocellular carcinoma treated with sorafenib tosylate showed massive tumor lysis with avoidance of tumor lysis syndrome. *Intern Med*. 2010;49(11):991-994. doi:10.2169/internalmedicine.49.3153
15. McBride A, Trifilio S, Baxter N, Gregory TK, Howard SC. Managing tumor lysis syndrome in the era of novel cancer therapies. *J Adv Pract Oncol*. 2017;8(7):705-720. doi:10.6004/jadpro.2017.8.7.4
16. Yasin AAH, Mohamed MIA, Magbol M, Izzoddeen A. Tumor lysis syndrome: frequency and outcome among acute leukaemia paediatric patients in Sudan, 2023. *Clin Med Res*. 2025;14(3):58-64. doi:10.11648/j.cmr.20251403.13
17. Friedman M, Patel PR, Rondelli D. A focused review of the pathogenesis, diagnosis, and management of tumor lysis syndrome for the interventional radiologist. *Semin Intervent Radiol*. 2015;32(2):231-236. doi:10.1055/s-0035-1549846
18. Mirrakhimov AE, Ali AM, Khan M, Barbaryan A. Tumor lysis syndrome in solid tumors: an up to date review of the literature. *Rare Tumors*. 2014;6(2):5389. doi:10.4081/rt.2014.5389
19. Pearson-Stuttard B, Soutar R, Leach M. Acute kidney injury from tumour lysis syndrome and urate crystalluria. *Br J Haematol*. 2015;170(4):444. doi:10.1111/bjh.13510
20. Canduri F, dos Santos DM, Silva RG, et al. Structures of human purine nucleoside phosphorylase complexed with inosine and ddI. *Biochem Biophys Res Commun*. 2004;313(4):907-914. doi:10.1016/j.bbrc.2003.11.179
21. Hsu HH, Lin JY, Hsu SC, Chan YL, Huang CC. Neglected cause of renal failure in cancer patients: spontaneous tumor lysis syndrome inducing acute uric acid nephropathy. *Dial Transplant*. 2004;33(6):316-325.
22. Cohen LF, Balow JE, Magrath IT, Poplack DG, Ziegler JL. Acute tumor lysis syndrome. a review of 37 patients with Burkitt's lymphoma. *Am J Med*. 1980;68(4):486-491. doi:10.1016/0002-9343(80)90286-7
23. Boles JM, Dutel JL, Briere J, et al. Acute renal failure caused by extreme hyperphosphatemia after chemotherapy of an acute lymphoblastic leukemia. *Cancer*. 1984;53(11):2425-2429. doi:10.1002/1097-0142(19840601)53:11<2425::aid-cncr2820531111>3.0.co;2-r
24. Dunlay RW, Camp MA, Allon M, Fanti P, Malluche HH, Llach F. Calcitriol in prolonged hypocalcemia due to the tumor lysis syndrome. *Ann Intern Med*. 1989;110(2):162-164. doi:10.7326/0003-4819-110-2-162
25. Shimada M, Johnson RJ, May WS Jr, et al. A novel role for uric acid in acute kidney injury associated with tumour lysis syndrome. *Nephrol Dial Transplant*. 2009;24(10):2960-2964. doi:10.1093/ndt/gfp330
26. Mirrakhimov AE, Voore P, Khan M, Ali AM. Tumor lysis syndrome: a clinical review. *World J Crit Care Med*. 2015;4(2):130-138. doi:10.5492/wjccm.v4.i2.130
27. Alhamid N, Sabbagh B, Alsarraj A, et al. Pitfalls of current diagnostic criteria of tumor lysis syndrome. *Kidney Blood Press Res*. 2024. doi:10.1159/000538328
28. Cairo MS, Bishop M. Tumour lysis syndrome: new therapeutic strategies and classification. *Br J Haematol*. 2004;127(1):3-11. doi:10.1111/j.1365-2141.2004.05094.x
29. Howard SC, Jones DP, Pui CH. The tumor lysis syndrome. *N Engl J Med*. 2011;364(19):1844-1854. doi:10.1056/NEJMra0904569
30. Lee AC. The use of single dose of rasburicase for the prophylaxis and treatment of tumor lysis syndrome in pediatric patients: a narrative review. *Hematol Rep*. 2025;17(6):71. doi:10.3390/hematolrep17060071
31. Tosi P, Barosi G, Lazzaro C, et al. Consensus conference on the management of tumor lysis syndrome. *Haematologica*. 2008;93(12):1877-1885. doi:10.3324/haematol.13290
32. Coiffier B, Altman A, Pui CH, Younes A, Cairo MS. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. *J Clin Oncol*. 2008;26(16):2767-2778. doi:10.1200/JCO.2007.15.0177
33. Xiao Y, Xiao L, Zhang Y, et al. Prediction of tumor lysis syndrome in childhood acute lymphoblastic leukemia based on machine learning models: a retrospective study. *Front Oncol*. 2024;14:1337295. doi:10.3389/fonc.2024.1337295
34. Conger JD, Falk SA. Intrarenal dynamics in the pathogenesis and prevention of acute urate nephropathy. *J Clin Invest*. 1977;59(5):786-793. doi:10.1172/JCI108700
35. Hagos Y, Stein D, Ugele B, Burckhardt G, Bahn A. Human renal organic anion transporter 4 operates as an asymmetric urate transporter. *J Am Soc Nephrol*. 2007;18(2):430-439. doi:10.1681/ASN.2006040415
36. Prasada H. Sevelamer hydrochloride for tumor lysis syndrome-related hyperphosphatemia. *Indian Pediatr*. 2015;52(7):613-615. doi:10.1007/s13312-015-0686-y
37. Worcester EM, Coe FL. Clinical practice. Calcium kidney stones. *N Engl J Med*. 2010;363(10):954-963. doi:10.1056/NEJMcp1001011
38. Spector T. Inhibition of urate production by allopurinol. *Biochem Pharmacol*. 1977;26(5):355-358. doi:10.1016/0006-2952(77)90191-5
39. LaRosa C, McMullen L, Bakdash S, et al. Acute renal failure from xanthine nephropathy during management of acute leukemia. *Pediatr Nephrol*. 2007;22(1):132-135. doi:10.1007/s00467-006-0287-z
40. Jeha S. Tumor lysis syndrome. *Semin Hematol*. 2001;38(4 Suppl 10):4-8. doi:10.1016/s0037-1963(01)90037-x
41. Keenan RT. Safety of urate-lowering therapies: managing the risks to gain the benefits. *Rheum Dis Clin North Am*. 2012;38(4):663-680. doi:10.1016/j.rdc.2012.08.008
42. Mayer MD, Khosravan R, Vernillet L, Wu JT, Joseph-Ridge N, Mulford DJ. Pharmacokinetics and pharmacodynamics of febuxostat, a new non-purine selective inhibitor of xanthine oxidase in subjects with renal impairment. *Am J Ther*. 2005;12(1):22-34. doi:10.1097/00045391-200501000-00005
43. Stamp LK. Safety profile of anti-gout agents: an update. *Curr Opin Rheumatol*. 2014;26(2):162-168. doi:10.1097/BOR.0000000000000031
44. Bellos I, Kontzoglou K, Psyrri A, Pergialiotis V. Febuxostat administration for the prevention of tumour lysis syndrome: a meta-analysis. *J Clin Pharm Ther*. 2019;44(4):525-533. doi:10.1111/jcpt.12839
45. Spina M, Nagy Z, Ribera JM, et al. FLORENCE: a randomized, double-blind, phase III pivotal study of febuxostat versus allopurinol for the prevention of tumor lysis syndrome (TLS) in patients with hematologic malignancies at intermediate to high TLS risk. *Ann Oncol*. 2015;26(10):2155-2161. doi:10.1093/annonc/mdv317
46. Bosly A, Sonet A, Pinkerton CR, et al. Rasburicase (recombinant urate oxidase) for the management of hyperuricemia in patients with cancer: report of an international compassionate use study. *Cancer*. 2003;98(5):1048-1054. doi:10.1002/cncr.11612
47. Lupușoru G, Ailincăi I, Frățilă G, et al. Tumor lysis syndrome: an endless challenge in onco-nephrology. *Biomedicines*. 2022;10(5):1012. doi:10.3390/biomedicines10051012

Ankyra Medical Journal

Case Report

DOI: 10.51271/ANKMJ-0067

# Phenotypic characterization of CRB1-associated Leber congenital amaurosis in two siblings: emphasis on para-arteriolar RPE preservation

**Hatice Zeynep İlikli***, **Tuğba Zeynep Göcen**, **Mehmet Yasin Teke**

*Department of Ophthalmology, Ankara Etlik City Hospital, Ankara, Turkiye*

**Cite this article:** İlikli HZ, Göcen TZ, Teke MY. Phenotypic characterization of CRB1-associated Leber congenital amaurosis in two siblings: emphasis on para-arteriolar RPE preservation. *Ank Med J.* 2026;5(2):56-60. doi: 10.51271/ANKMJ-0067

**Received**: 20/10/2025 ◆ **Accepted**: 01/12/2025 ◆ **Published**: 25/03/2026

## ABSTRACT

Leber congenital amaurosis (LCA) associated with CRB1 mutations exhibits a distinct retinal phenotype, most notably para-arteriolar retinal pigment epithelium preservation (PPRPE), an important yet often under-recognized diagnostic hallmark. We report two affected female siblings from a consanguineous family who underwent comprehensive multimodal ophthalmic evaluation, including best-corrected visual acuity assessment, color fundus photography, spectral-domain optical coherence tomography (OCT), fundus autofluorescence (FAF), and full-field electroretinography (ERG). Both demonstrated congenital severe visual impairment and nystagmus. Fundus examination revealed bilateral nummular pigmentation with characteristic PPRPE, while FAF showed para-arteriolar hyperautofluorescence consistent with preserved RPE integrity. OCT demonstrated diffuse retinal thinning, macular disorganization, and peripheral atrophy, and ERG responses were markedly reduced or extinguished. Next-generation sequencing identified a homozygous pathogenic CRB1 variant, c.2843G>A, p.(Cys948Tyr) (NM_201253), classified as pathogenic according to ACMG-2015 criteria, confirming CRB1-associated LCA. Additional affected male family members further supported autosomal recessive inheritance. The coexistence of PPRPE, macular structural abnormalities, and severe electrophysiological dysfunction aligns with the established CRB1 phenotypic spectrum. These findings reinforce the diagnostic utility of PPRPE in differentiating CRB1-associated LCA from other molecular subtypes and underscore the importance of integrating multimodal imaging with early molecular testing to facilitate accurate diagnosis, targeted genetic counseling, and potential future eligibility for emerging genotype-directed therapies.

**Keywords:** Leber congenital amaurosis, CRB1 gene, para-arteriolar retinal pigment epithelium, optical coherence tomography, fundus autofluorescence

## INTRODUCTION

Leber congenital amaurosis (LCA) represents the most severe form of inherited retinal dystrophy presenting in early infancy and is genetically heterogeneous, with more than twenty genes implicated to date.[1] Among these, CRB1 is a well-established cause of LCA and early-onset severe retinal dystrophy (EOSRD), accounting for approximately 10% of cases in large cohorts.[2,3] Pathogenic variants in CRB1 disrupt the Crumbs complex, a critical regulator of photoreceptor polarity, retinal lamination, and Müller cell integrity.[4,5] As a result, CRB1-associated retinopathies exhibit a distinct constellation of structural abnormalities, including coarse retinal lamination, thickened retina in early disease stages, and progressive macular disorganization.[2,6]

One of the hallmark clinical features of CRB1-associated LCA is para-arteriolar retinal pigment epithelium preservation (PPRPE), a striking and highly characteristic fundus sign first recognized more than two decades ago.[7] PPRPE reflects selective preservation of RPE along retinal arterioles despite widespread peripheral atrophy and is increasingly recognized as a valuable diagnostic clue guiding early genotypic suspicion.[2,7,8] Recent studies highlight its utility in differentiating CRB1-associated LCA from other genetic subtypes such as CEP290 or RPE65, which lack this distinctive pattern.[9,10] However, despite its diagnostic value, PPRPE remains underreported and may be overlooked without multimodal imaging techniques such as fundus autofluorescence (FAF) and optical coherence tomography (OCT).[6,8]

Multimodal imaging plays a central role in the phenotyping of CRB1-associated retinopathies. FAF often demonstrates para-arteriolar hyper autofluorescence consistent with preserved RPE integrity, whereas OCT reveals inner retinal disorganization, altered retinal lamination, and progressive atrophy.[6,8,11] Full-field electroretinography (ERG) typically

**Corresponding Author:** Hatice Zeynep İlikli, zilikli_98@hotmail.com

shows severely reduced or extinguished responses, reflecting widespread photoreceptor dysfunction.[12] The integration of these imaging modalities with next-generation sequencing (NGS) enables precise genotype–phenotype correlation and informs genetic counseling and emerging gene-based therapeutic strategies.[13,14]

Here, we present two affected female siblings from a consanguineous family with genetically confirmed CRB1-associated LCA. We emphasize the diagnostic relevance of PPRPE supported by multimodal imaging and highlight the clinical features, electrophysiological characteristics, and molecular findings that contribute to accurate diagnosis and improved understanding of the CRB1-LCA phenotype.

## CASE

Two affected female siblings from a consanguineous family were referred to our inherited retinal disease clinic for evaluation of lifelong visual impairment. Both individuals exhibited symptoms consistent with congenital onset LCA, including poor visual behavior in infancy and early nystagmus. A detailed family history revealed additional affected male relatives on the paternal side, supporting an autosomal recessive inheritance pattern.

### Patient 1

A 31-year-old female reported stable but severely reduced visual acuity since infancy. Best-corrected visual acuity (BCVA) was 20/200 in right eye and counting fingers at 1 meter in the left eye. Anterior segment examination was unremarkable. Fundus photography showed bilateral nummular pigmentary deposits, attenuated retinal vessels and prominent PPRPE (Figure 1). FAF, obtained using the Zeiss Visucam platform, demonstrated para-arteriolar hyperautofluorescence corresponding to preserved RPE integrity, surrounded by peripheral hypo autofluorescent atrophic zones (Figure 2). Both patients exhibited high hyperopia and normal intraocular pressures (14-15 mmHg).

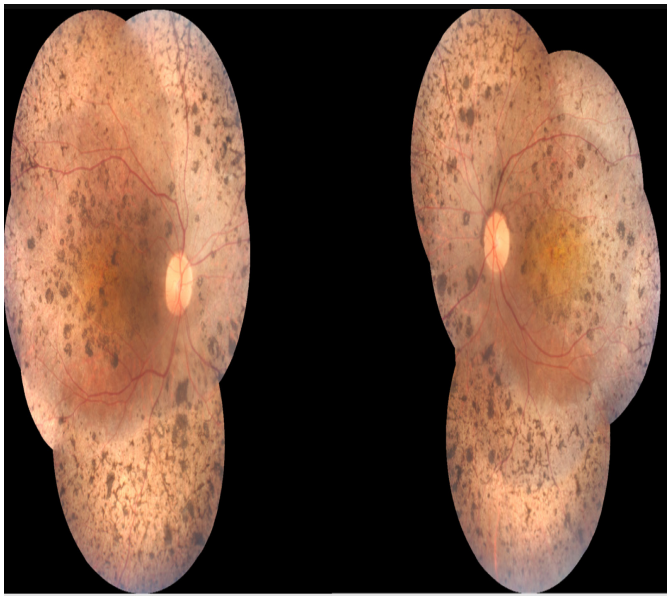

**Figure 1.** Color fundus photograph of the 31-year-old female sibling
Color fundus photography showing para-arteriolar RPE preservation (PPRPE), nummular pigmentation, vascular attenuation

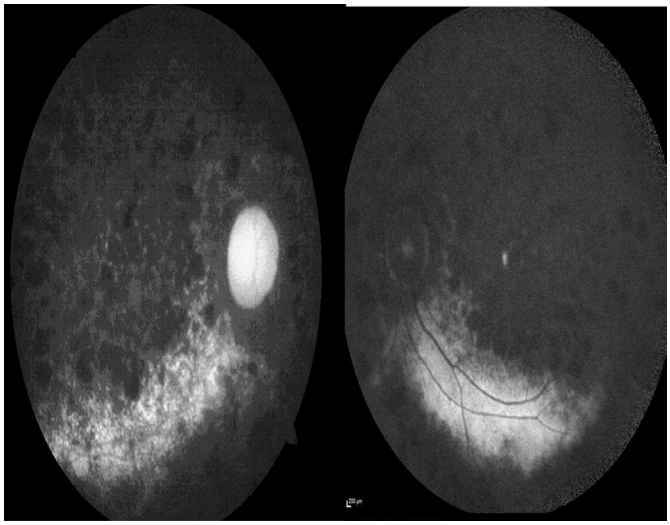

**Figure 2**. Fundus autofluorescence demonstrating para-arteriolar hyper autofluorescence with peripheral hypo autofluorescence

Spectral-domain optical coherence tomography (SD-OCT; Carl Zeiss Stratus OCT III) revealed diffuse retinal thinning, disruption of the outer retinal layers, and central macular disorganization with loss of the photoreceptor IS/OS junction (Figure 3). Full-field ERG; Metrovision Mon 2021A, ISCEV-standard showed extinguished scotopic and photopic responses, indicating severe global retinal dysfunction.[12]

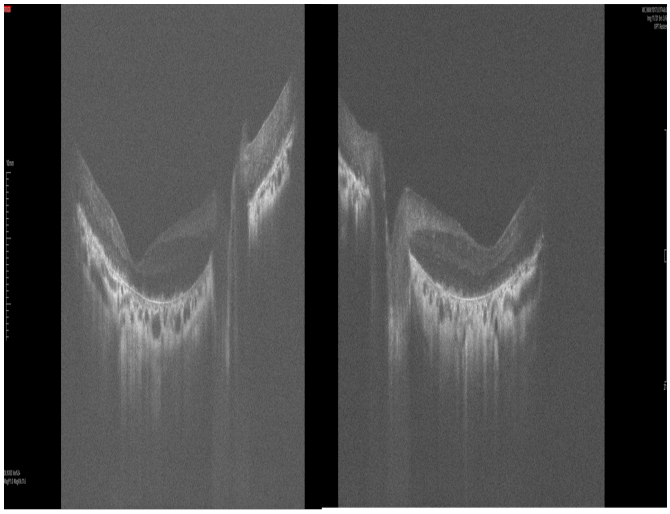

**Figure 3**. Spectral-domain optical coherence tomography (SD-OCT) of the elder sister
OCT demonstrating Diffuse retinal thinning, foveal contour distortion, and loss of outer retinal layers.

### Patient 2

A 19-year-old female sibling reported similar congenital visual impairment. BCVA measured 20/400 bilaterally. The younger sibling demonstrated horizontal nystagmus, while ocular motility and anterior segment examination were otherwise unremarkable. Fundus examination demonstrated a nearly identical phenotype, including bilateral PPRPE, nummular pigmentation, vascular attenuation, and optic disc drusen (Figure 4). FAF imaging showed para-arteriolar hyper autofluorescence with more pronounced central hypo autofluorescence compared with her sister (Figure 2). SD-OCT demonstrated advanced retinal thinning, laminar disorganization, and perifoveal atrophy (Figure 5). ERG responses were extinguished across all modalities.

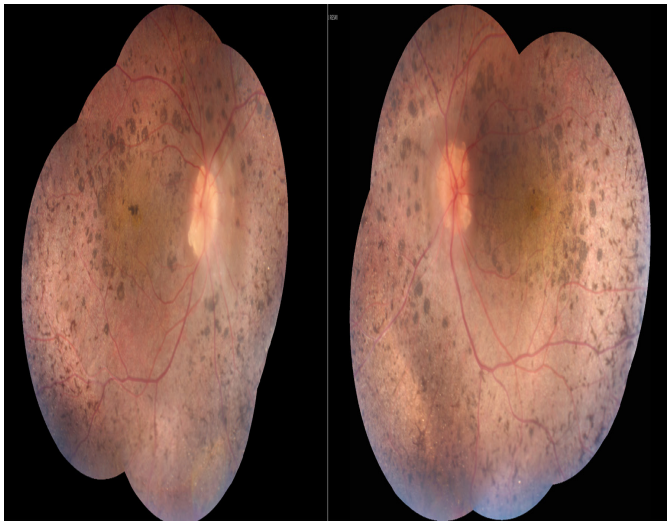

**Figure 4.** Colour fundus photograph of the 19-year-old female sibling
Color fundus photography showing para-arteriolar RPE preservation (PPRPE), nummular pigmentation, vascular attenuation and optic disc drusen

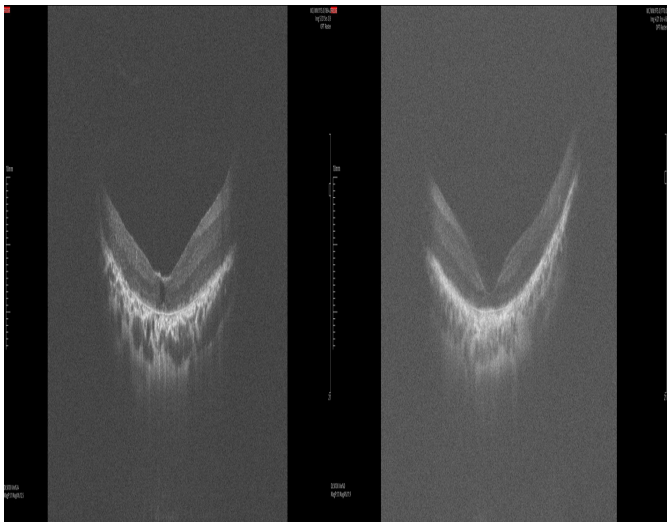

**Figure 5.** Spectral-domain optical coherence tomography (SD-OCT) of the younger sister
OCT demonstrating more advanced thinning, laminar disorganization, and perifoveal atrophy

## Genetic Findings

Both siblings underwent targeted NGS using a 41+ gene inherited retinal disease (IRD) panel on the Illumina sequencing platform. Sequencing reads were aligned to the GRCh37/hg19 reference genome. The assay achieved mean coverage >100× and >99% of coding regions covered at ≥20×, meeting standards for IRD molecular diagnostics.[3,13]

NGS revealed a homozygous c.2843G>A, p.(Cys948Tyr) pathogenic variant in CRB1 (NM_201253), classified as pathogenic according to ACMG-2015 criteria. The variant is previously reported in association with CRB1-LCA and demonstrated high pathogenicity on in-silico analysis (CADD, PolyPhen-2, MutationTaster). Genetic findings confirmed CRB1-associated LCA in both siblings.

Based on clinical, electrophysiological and genetic findings, a diagnosis of CRB1-related LCA was made. Both patients were referred for low-vision rehabilitation and supportive educational services. They were also counseled regarding potential participation in emerging gene therapy clinical trials for CRB1-associated retinal dystrophies. (A detailed summary of clinical imaging and genetic findings is provided in Table.)

# DISCUSSION

LCA associated with CRB1 mutations represents a distinct clinical and structural phenotype within the spectrum of early-onset inherited retinal dystrophies. The two affected siblings presented here demonstrate several hallmark features of CRB1-associated disease, including congenital severe visual impairment, extinguished electroretinographic responses, PPRPE, and characteristic multimodal imaging abnormalities. Their findings closely parallel the phenotypic patterns reported in large CRB1 cohorts.[2,6,8]

PPRPE has long been recognized as a distinctive hallmark of CRB1-related retinopathies.[7] Mechanistically, several hypotheses have been proposed. First, para-arteriolar regions

**Table.** Clinical, imaging, and genetic characteristics of the two affected siblings

| Parameter | Sibling 1 (age 31) | Sibling 2 (age 19) |
|---|---|---|
| **Sex** | **Female** | **Female** |
| Family history | Consanguineous parents; additional affected males | Consanguineous parents; additional affected males |
| Onset | Congenital | Congenital |
| BCVA (Snellen) | 20/200 OD<br>20/400 OS | 20/400 OU |
| Anterior segment | Normal | Horizontal nistagmus |
| Fundus findings | Nummular pigmentation; vessel attenuation; PPRPE | Nummular pigmentation; vessel attenuation; PPRPE; optic disc drusen; more pronounced pigmentary changes |
| FAF | Para-arteriolar hyper autofluorescence with peripheral hypo-AF | Identical pattern; more central hypo-AF |
| SD-OCT | Diffuse thinning; macular disorganization; outer retinal loss | Severe thinning; laminar disorganization; perifoveal atrophy |
| ERG | Severely reduced/extinguished | Extinguished |
| VEP | Delayed latency, reduced amplitude | Delayed latency, reduced amplitude |
| Genetic testing | Homozygous CRB1 c.2843G>A, p.(Cys948Tyr) | Homozygous CRB1 c.2843G>A, p.(Cys948Tyr) |
| NGS panel coverage | Mean depth >100×; >99% bases ≥20× | Mean depth >100×; >99% bases ≥20× |
| Final diagnosis | CRB1-associated LCA | CRB1-associated LCA |

BCVA: Best-corrected visual acuity, FAF: Fundus autofluorescence, SD-OCT: Spectral-domain optical coherence tomography, ERG: Electroretinography, PPRPE: Para-arteriolar retinal pigment epithelium preservation, LCA: Leber congenital amaurosis, VEP: Visual evoked potential, NGS: Next-generation sequencing

may exhibit relative RPE and Müller cell resilience, supported by differential metabolic demand or structural support along arterioles.[5] Second, oxygen tension may be comparatively higher adjacent to retinal arterioles, preserving RPE viability in these zones despite widespread degeneration.[15] Third, CRB1—a critical component of the Crumbs complex—helps maintain retinal epithelial polarity and adherens junctions, and its dysfunction may lead to selective RPE vulnerability except in areas with compensatory Müller cell support.[4] These mechanistic models collectively explain why PPRPE remains one of the most reliable clinical markers of CRB1 involvement.

The value of PPRPE is further underscored when differentiating CRB1-LCA from other genotypes. In contrast to CRB1 disease, RPE65-associated LCA typically exhibits widespread early RPE dysfunction without para-arteriolar sparing.[10] Likewise, CEP290-LCA often demonstrates severe foveal hypoplasia and preserved retinal architecture in infancy but does not show PPRPE.[9] Thus, PPRPE represents an important diagnostic clue, enabling clinicians to prioritize CRB1 analysis early in the diagnostic pathway.

The prognostic significance of PPRPE remains a subject of ongoing study. While some authors have suggested that areas of preserved RPE may correlate with localized structural stability,[6,7] current evidence does not support a clear association between the presence of PPRPE and slower global disease progression. Phenotypic severity among CRB1 patients is highly variable—even in individuals carrying the same variant.[2,8] Our two siblings, both exhibiting PPRPE, nevertheless demonstrated advanced structural and functional deterioration, reinforcing that PPRPE should be regarded primarily as a diagnostic, rather than prognostic, feature.

Genotype–phenotype relationships in CRB1-associated disease are similarly complex. Studies suggest that loss-of-function variants often produce more severe phenotypes with early retinal disorganization,[2,4] whereas missense variants, including the p.(Cys948Tyr) substitution identified in our patients, may result in a broader clinical spectrum with some retention of para-arteriolar RPE integrity.[8,11] The variant detected in this family has been previously reported and is consistently classified as pathogenic. The shared homozygous genotype in both siblings, combined with multiple affected family members, supports autosomal recessive inheritance consistent with prior CRB1 reports.

Multimodal imaging serves as an indispensable tool for characterizing CRB1-associated LCA. FAF remains particularly sensitive in visualizing PPRPE, and SD-OCT reveals essential structural correlates, including inner retinal disorganization, thickened retinal layers in early stages, and progressive thinning with age.[8,16] ERG findings in our patients—severely reduced or extinguished responses—align with the advanced disease stage expected in adult CRB1-LCA.[12] The combination of multimodal imaging and NGS has become the cornerstone of accurate diagnosis and genotype–phenotype correlation in LCA, aligning with current consensus recommendations.[13]

As emerging gene-based therapies continue to expand for inherited retinal dystrophies, accurate molecular diagnosis of CRB1-associated disease is increasingly important. Although no approved CRB1-targeted therapy exists, several preclinical programs—including AAV-based gene augmentation and CRISPR-mediated editing—are in development.[14] Identifying affected families with confirmed pathogenic variants is therefore essential for future clinical trial eligibility.

## CONCLUSION

This study describes two affected female siblings with genetically confirmed CRB1-associated LCA, demonstrating the classic phenotypic triad of PPRPE, macular structural abnormalities, and severe electrophysiological dysfunction. The findings reinforce the utility of multimodal imaging for early clinical suspicion and highlight the importance of integrating molecular diagnostics for accurate genotypic confirmation. PPRPE remains a key distinguishing feature of CRB1-associated disease, although its prognostic significance is limited. As gene-based therapeutic strategies for CRB1-associated retinal dystrophies advance, early and precise diagnosis will become increasingly critical for counseling, family screening, and treatment eligibility.

## ETHICAL DECLARATIONS

### Informed Consent

Written informed consent was obtained from the patients included in this report. Signed consent forms are retained by the authors and are available upon request.

### Peer Review Process

This report underwent external peer review.

### Conflict of Interest

The authors declare no conflicts of interest.

### Financial Disclosure

This case report did not receive any financial support.

### Author Contributions

Concept: HZI; Design: HZI; Control: HZI, MYT; Resources: HZI, TZG, MYT; Materials: HZI, TZG; Data Collection and/or Processing: HZI, TZG; Analysis and/or Interpretation: HZI, MYT; Literature Review: HZI, TZG; Writing the Article: HZI, TZG, MYT; Critical Review: HZI, TZG, MYT.

### Acknowledgments

The authors would like to thank the ophthalmology team at Ankara Etlik City Hospital for their support during the clinical evaluations.

## REFERENCES

1. den Hollander AI, Roepman R, Koenekoop RK, Cremers FPM. Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog Retin Eye Res.* 2008;27(4):391-419. doi:10.1016/j.preteyeres.2008.05.003
2. Bujakowska KM, Audo I, Mohand-Saïd S, et al. CRB1 mutations in inherited retinal dystrophies. *Prog Retin Eye Res.* 2012;31(2):120-147. doi:10.1016/j.preteyeres.2012.01.001
3. Stone EM. Leber congenital amaurosis—a model for efficient genetic testing via NGS panels. *Ophthalmology.* 2017;124(7):1062-1064. doi:10.1016/j.ophtha.2017.03.001
4. Pellissier LP, Alves CH, Quinn PM, et al. Targeted disruption of the CRB1 gene in mice reproduces the retinal phenotype associated with CRB1 mutations in humans. *Hum Mol Genet.* 2013;22(1):35-48. doi:10.1093/hmg/dds399

5. Alves CH, Pellissier LP, Vos RM, et al. CRB1: A master regulator of photoreceptor–Müller glia interaction. *Hum Mol Genet.* 2014;23(20): 5359-5370. doi:10.1093/hmg/ddu251
6. Galvin JA, Fishman GA, Stone EM, et al. CRB1-associated retinal dystrophies: clinical and imaging characteristics. *Retina.* 2020;40(3): 469-480. doi:10.1097/IAE.0000000000002372
7. den Hollander AI, Heckenlively JR, van den Born LI, et al. CRB1 mutations and para-arteriolar pigment preservation in LCA. *J Med Genet.* 2001;38(2):109-113. doi:10.1136/jmg.38.2.109
8. Nguyen X-T, Birtel J, Gliem M, et al. Imaging features of CRB1-LCA: a multimodal approach. *Ophthalmic Genet.* 2022;43(4):440-450. doi:10. 1080/13816810.2022.2070697
9. Cideciyan AV, Aleman TS, Jacobson SG, et al. CEP290-LCA: Imaging and functional phenotype. *Invest Ophthalmol Vis Sci.* 2007;48(9):4001-4009. doi:10.1167/iovs.07-0412
10. Jacobson SG, Cideciyan AV, Roman AJ, et al. Natural history of RPE65-LCA. *Ophthalmology.* 2020;127(10):1393-1404. doi:10.1016/j.ophtha. 2020.03.012
11. Talib M, van Schooneveld MJ, van Genderen MM, et al. Structural OCT characteristics in CRB1-linked early-onset retinal dystrophy. *Br J Ophthalmol.* 2017;101(5):627-634. doi:10.1136/bjophthalmol-2016- 309376
12. McCulloch DL, Marmor MF, Brigell MG, et al. ISCEV Standard for clinical electroretinography. *Doc Ophthalmol.* 2022;144(3):209-245. doi: 10.1007/s10633-022-09864-5
13. Consugar MB, Navarro-Gomez D, Place EM, et al. Clinical NGS testing in hereditary retinal disorders. *Hum Mutat.* 2015;36(6):591-602. doi:10. 1002/humu.22781
14. Kumaran N, Moore AT, Weleber RG, et al. Gene therapy prospects for CRB1-associated retinal dystrophies. *Hum Gene Ther.* 2020;31(11-12): 702-716. doi:10.1089/hum.2020.29020.nku
15. Quinn N, Csincsik L, Flynn E, et al. CRB1-associated retinal dystrophies: expanding phenotypes. *Ophthalmology.* 2019;126(11):1546-1558. doi:10.1016/j.ophtha.2019.05.012
16. Chen Y, Zeng C, Wang C, et al. Structural–functional correlation in CRB1 retinopathy. *Retina.* 2019;39(6):1171-1181. doi:10.1097/IAE. 0000000000002100

Ankyra Medical Journal

Letter to the Editor DOI: 10.51271/ANKMJ-0068

# Unexpected etiology of massive lower gastrointestinal bleeding requiring blood transfusion: Crohn's disease, syphilis, or hematological malignancy?

Cansın Taşkın, Alpaslan Tanoğlu*

*Department of Internal Medicine, Faculty of Medicine, Bahçeşehir University, İstanbul, Turkiye*

**Cite this article:** Taşkın C, Tanoğlu A. Unexpected etiology of massive lower gastrointestinal bleeding requiring blood transfusion: Crohn's disease, syphilis, or hematological malignancy? *Ank Med J.* 2026;5(2):61-62. doi: 10.51271/ANKMJ-0068

**Received**: 29/12/2026 ◆ **Accepted**: 08/02/2026 ◆ **Published**: 25/03/2026

**Keywords:** Crohn's disease, lower gastrointestinal bleeding, syphilis, hematological malignancy

**Dear Editor,**

Crohn's disease (CD) is a chronic inflammatory bowel disease that may involve any segment of the gastrointestinal (GI) tract and typically presents with abdominal pain, diarrhea, weight loss, and occasionally hematochezia. CD is difficult to diagnose and can be confused with intestinal involvement in various infections, including tuberculosis.[1-4] Although mild GI bleeding is relatively common, massive lower GI bleeding requiring transfusion is rare[5] and should prompt careful reconsideration of the initial diagnosis and evaluation for alternative or concomitant etiologies. Here, we present a patient initially diagnosed with CD who developed life-threatening lower GI bleeding, ultimately found to have infectious and hematological conditions contributing to the clinical picture.

A 60-year-old male presented to the emergency department with recurrent bloody defecation and lethargy. The patient appeared pale and was anxious. His medical history included hypertension. One week prior to admission, the patient had been evaluated for mucoid diarrhea and underwent colonoscopy, after which a diagnosis of CD was established and he was given azathioprine and prednisolone therapy by another gastroenterologist. Physical examination revealed normal cardiac and pulmonary functions. Cervical and axillary lymph nodes were palpated. Upon admission his blood pressure was 100/60 mmHg and his pulse was 86/minute. His initial laboratory evaluation revealed anemia and leukocytosis (hemoglobin level of 8.1 g/dl, hematocrit: 24.5%, WBC: 17.55 K/uL, monocyte count: 6,48 K/uL). Prothrombin time and C-reactive protein levels were within normal limits. Routine laboratory tests were within normal reference ranges except elevated urea (64 mg/dl) and albumin levels (3.2 g/dl). Because of lower GI bleeding, he was hospitalized, oral intake was stopped and transfused with two units of packed red blood cells. The following day, a colonoscopy was performed, which revealed deep ulcerations in the descending and transverse colon, accompanied by two large adjacent clot formations (Figure 1). No active bleeding focus was identified at that time. Despite initial supportive management, the patient continued to pass bloody stools, necessitating transfusion of an additional four units of packed red blood cells. Because of this clinical picture, CT angiography of the abdomen was performed and it suggested an active bleeding source at the junction of the splenic flexure and transverse colon. A repeated colonoscopy again failed to localize an active bleeding site due to the presence of heavy blood contamination and blood clots. Immediately interventional radiology team was called to the hospital and two active bleeding vessels were successfully embolized by them, resulting in hemodynamic stabilization and cessation of overt GI bleeding. Given the patient's persistent monocytosis, which had been documented also prior to admission, low hemoglobin levels and the presence of generalized lymphadenopathy, and also he was consulted by the infectious diseases department. His serological testing revealed Bartonella henselae IgM positivity and a positive *Treponema pallidum* hemagglutination assay (TPHA). Azithromycin 250 mg P.O. once daily was given for 2 weeks for *Bartonella henselae* infection without organ involvement and to treat syphilis weekly 2.400 000 IU Penicillin G benzathine IM injections were initiated. Peripheral blood smear examination demonstrated dysplastic monocytes and approximately 10% circulating blasts. Subsequent bone marrow biopsy confirmed the diagnosis of acute myeloid leukemia (AML) with FLT3 mutation positivity, and the patient was referred to a bone marrow transplantation center for definitive management. In line with these clinical developments, the patient's initial pathology specimens were obtained and sent to an external center for examination by a pathologist experienced in the diagnosis of inflammatory bowel diseases. The pathologist's examination indicated that the pathology samples were not consistent with CD. The patient presented to our hospital with a bleeding disorder,

**Corresponding Author:** Alpaslan Tanoğlu, alpaslantanoglu@yahoo.com

so azathiopurin treatment was discontinued, and steroid treatment was also stopped by tapering due to the pathology preparations not being consistent with CD. Based on these developments, it was assessed that the lower GI bleeding was caused by syphilis or AML. After discontinuing the treatment for CD and using antibiotics for the infections, the patient's GI symptoms improved. A follow-up colonoscopy performed 4 months after the patient's emergency room visit, while still in remission from AML, was found to be completely normal (Figure 2).

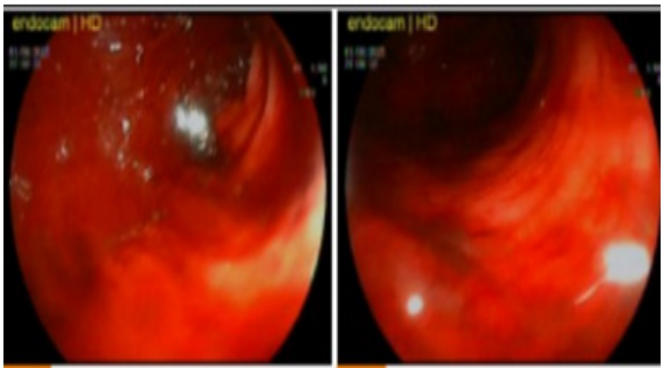


**Figure 1.** Endoscopic appearance of the colonic bleeding in descending and sigmoid colon

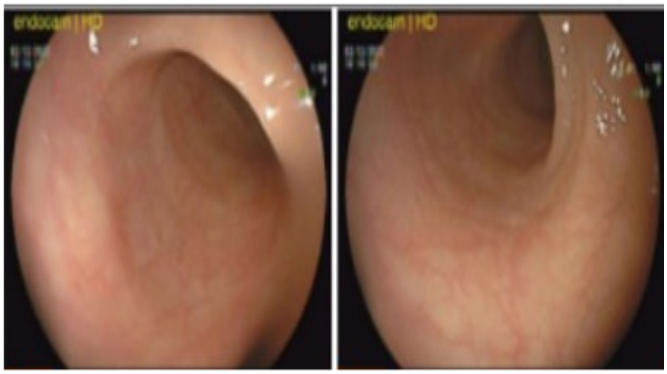


**Figure 2.** Follow-up colonoscopy four months later, showing complete mucosal healing with a normal colonic appearance

This case indicates several important clinical considerations. Although CD may rarely cause severe GI bleeding, deep ulcerations and transfusion-dependent hemorrhage should raise suspicion for alternative or additional pathologies. Infectious agents such as *Bartonella* species may involve the GI tract, particularly in immunosuppressed individuals,[6] and may exacerbate mucosal injury causing mucosal ulcerations in the GI tract.[6,7] Furthermore, ulcerations along the GI tract and lower GI bleeding caused by *Treponema pallidum* is documented and presented as literature information.[6,8,9] Hematologic malignancies such as AML can also contribute to GI bleeding[10] through mucosal fragility, leukemic infiltration, thrombocytopenia, and coagulopathy. Importantly, the initiation of immunosuppressive therapy based on an initial diagnosis of CD may have facilitated progression or unmasking of underlying infectious and malignant conditions in this patient. Therefore, clinicians should maintain a high index of suspicion for secondary causes in patients with presumed inflammatory bowel disease who exhibit atypical features such as persistent monocytosis, generalized lymphadenopathy, extensive deep ulcerations, or refractory and massive GI bleeding. In conclusion, this case highlights the critical importance of comprehensive reassessment in patients with an established diagnosis of CD who demonstrate an unusual or aggressive clinical course. Early consideration of infectious and hematological etiologies may prevent diagnostic delay and guide appropriate, potentially life-saving interventions.

## ETHICAL DECLARATIONS

### Informed Consent

Written informed consent was obtained from the patient for the publication of this correspondence and any related clinical details.

### Peer Review Process

This letter was externally peer-reviewed.

### Conflict of Interest

The authors declare no conflicts of interest.

### Financial Disclosure

No financial support was received for the preparation or publication of this letter.

### Author Contributions

Concept: CT, AT; Design: AT;Control: CT, AT; Data Collection and/or Processing: CT,AT; Analysis and/or Interpretation: CT, AT; LiteratureReview: CT, AT; Article Writing: CT, AT; Critical Review:All Authors.

## REFERENCES

1. Kekilli M, Tanoğlu A, Karaahmet F, et al. Midkine level may be used as a noninvasive biomarker in Crohn's disease. *Turk J Med Sci.* 2020;50(2):324-329. doi:10.3906/sag-1904-167
2. Onal IK, Kekilli M, Tanoglu A, Erdal H, Ibis M, Arhan M. Tuberculosis and Crohn's disease revisited. *J Coll Physicians Surg Pak*. 2015;25(6):443-448.
3. Kirkik D, Kalkanli Tas S, Tanoglu A. Unraveling the blood microbiome: novel insights into inflammasome responses in Crohn's disease. *Eur J Gastroenterol Hepatol.* 2024;36(8):975-984. doi:10.1097/MEG.0000000000002695
4. Yang X, Guo H, Zou M. Inflammatory bowel diseases: pathological mechanisms and therapeutic perspectives. *Mol Biomed.* 2026;7(1):2. doi:10.1186/s43556-025-00395-z
5. Podugu A, Tandon K, Castro FJ. Crohn's disease presenting as acute gastrointestinal hemorrhage. *World J Gastroenterol.* 2016;22(16):4073-4078. doi:10.3748/wjg.v22.i16.4073
6. Mosepele M, Mazo D, Cohn J. Bartonella infection in immunocompromised hosts: immunology of vascular infection and vasoproliferation. *Clin Dev Immunol.* 2012;2012:612809. doi:10.1155/2012/612809
7. Wang KR, Li Z, Li YQ. Gastrointestinal ulcers in a patient with a solitary enlarged lymph node. *Gastroenterology.* 2019;157(4):e10-e11. doi:10.1053/j.gastro.2019.05.052
8. Ferzacca E, Barbieri A, Barakat L, Olave MC, Dunne D. Lower gastrointestinal syphilis: case series and literature review. *Open Forum Infect Dis.* 2021;8(6):ofab157. doi:10.1093/ofid/ofab157
9. Düzenli T, Kaplan M, Tanoğlu A, Salmanoğlu M. An unusual method to diagnose a rare disease: upper gastrointestinal endoscopy. *Turk J Gastroenterol.* 2017;28(6):522-523. doi:10.5152/tjg.2017.17224
10. Sobotka LA, Crilley T, Levine E, Afzali A. Acute myelogenous leukemia as a rare cause of duodenal ulcers. *ACG Case Rep J.* 2019;6(7):e00153. doi:10.14309/crj.0000000000000153