



HAL
open science

Hyper-alkalinization without hyper-hydration for the prevention of high-dose methotrexate acute nephrotoxicity in patients with osteosarcoma

Olivier Mir, Stanislas Ropert, Antoine Babinet, Jérôme Alexandre, Frédérique Larousserie, Jean-Philippe Durand, Eric Enkaoua, Philippe Anract, François Goldwasser

► To cite this version:

Olivier Mir, Stanislas Ropert, Antoine Babinet, Jérôme Alexandre, Frédérique Larousserie, et al.. Hyper-alkalinization without hyper-hydration for the prevention of high-dose methotrexate acute nephrotoxicity in patients with osteosarcoma. *Cancer Chemotherapy and Pharmacology*, 2010, 66 (6), pp.1059-1063. 10.1007/s00280-010-1259-3 . hal-00568280

HAL Id: hal-00568280

<https://hal.science/hal-00568280>

Submitted on 23 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Original article

changes are marked in yellow

Hyper-alkalinization without hyper-hydration for the prevention of high-dose methotrexate acute nephrotoxicity in osteosarcoma patients.

Olivier Mir^a, Stanislas Ropert^a, Antoine Babinet^b, Jérôme Alexandre^a, Frédérique Larousserie^c, Jean-Philippe Durand^a, Eric Enkaoua^d, Philippe Anract^b, and François Goldwasser^a.

Departments of ^aMedical Oncology, ^bOrthopaedic Surgery and ^cPathology, Université Paris Descartes, Assistance Publique – Hôpitaux de Paris, Teaching Hospital Cochin, Paris, France, and ^dDepartment of Orthopaedic Surgery, Université Pierre et Marie Curie, Assistance Publique – Hôpitaux de Paris, Teaching Hospital Pitié-Salpêtrière, Paris, France.

Corresponding author :

Olivier Mir

Université Paris Descartes, Assistance Publique – Hôpitaux de Paris

Department of Medical Oncology, Teaching Hospital Cochin,

27, rue du faubourg Saint-Jacques F75014 Paris, France.

Tel : + 33 6 30 73 90 79

Fax : + 33 1 58 41 17 45

E-mail : olivier.mir@cch.aphp.fr

The Authors declare no conflicts of interest.

Disclosures: none.

Words: 1934

Tables: 4

Figures: 0

Abstract (204 words)

Purpose: To evaluate the reliability and renal safety of an original schedule of high-dose methotrexate (HDMTX) administration with hyper-alkalinization, and without hyper-hydration.

Methods: Osteosarcoma patients received HDMTX (8-12 g/m²) as a 4-hour infusion. Hypertonic 8.4% sodium bicarbonate was infused prior to HDMTX, then once daily for 3 days. Methotrexate serum concentrations was measured at hour 4 (C_{max}), hour 24, hour 48 and hour 72. Urinary pH was measured on each miction. Serum creatinine was assessed on days 1, 3 and 8.

Results: Twenty-six patients (median age: 18 years, range: 15-25) received a total of 344 cycles of HDMTX, including 16 patients treated in an outpatient basis. Urinary pH remained constantly higher than 7.5 in all patients. Grade 1 creatininemia toxicity was observed in 31 cycles (9%), and grade 2 creatinine toxicity was observed in one patient. No episode of acute severe nephrotoxicity was observed. No significant worsening was observed in serum creatinine and calculated creatinine clearance from baseline to the end of therapy (p = 0.74). The main extra-renal toxicity was alkalinization-related hypokalemia from H48. No re-hospitalisation was required.

Conclusion: Hyper-alkalinization appears an efficient and reliable method to prevent the acute renal toxicity of HDMTX, and allows its safe administration in the outpatient setting.

Key words: pharmacokinetics, high-dose methotrexate, nephrotoxicity, outpatient, osteosarcoma, sarcoma.

Introduction

Osteosarcoma (OS) is a rare malignant tumour that mainly afflicts children and young adults. Prior to the introduction of systemic chemotherapy, approximately 80%-90% of patients died despite early radical surgery [1]. Neoadjuvant chemotherapy dramatically improved the disease-free survival and overall survival for OS patients, and is considered the gold standard treatment modality [2,3].

The most effective drugs in OS are: cisplatin, doxorubicin, ifosfamide and high-dose methotrexate (HDMTX) [4,5]. These drugs are always administered sequentially, because HDMTX-induced nephrotoxicity makes its administration with cisplatin or ifosfamide at high risk. The standard administration of HDMTX includes hyper-hydration, typically: 2.5-3.5 l/m²/24h (beginning 12h before HDMTX infusion, for 36-48h). Close monitoring of urinary pH is mandatory, with administration sodium bicarbonate before and after the infusion (45-50 mEq per liter of IV fluids), in order to maintain alkaline urinary pH [6]. However, this standard protocol shows several limitations. Firstly, hyper-hydration may obscure the ability to reach the threshold peak [7,8] and AUC (area under the concentration vs. time curve) [9] required to ensure the efficacy of HDMTX in OS. Hence, hyper-hydration rapidly decreases the plasma-to-tissue concentration gradient of methotrexate (MTX) and might reduce its anti-tumoral effect [10,11]. Secondly, the *a posteriori* administration of oral or IV sodium bicarbonate does not reverse the renal damage caused by HDMTX in all cases, and a sizeable proportion of patients develop progressive renal function impairment after HDMTX-based chemotherapy [12]. Thirdly, Sand et al. [13] showed that maintaining an alkaline urinary pH was more important than maintaining high urinary flow to obtain optimal HDMTX clearance.

Finally, a close fortnightly monitoring of urinary pH requires careful nursing care, as well as the cooperation of patients that may be difficult to obtain.

In view of these limitations, a strong and preventive - instead of *a posteriori* - urine alkalinization would be a coherent approach in view of the pathogenesis of HDMTX induced acute nephrotoxicity. Indeed, HDMTX-induced nephrotoxicity is mainly mediated by the precipitation of MTX and its metabolites in the renal tubules [14]. The solubility of MTX and its metabolites is closely related to urinary pH (Table 1) [15].

As a consequence, we hypothesized that: i) an aggressive strategy of preventive urine alkalinization could prevent HDMTX-induced acute nephrotoxicity; ii) renal safety provided by hyper-alkalinization could allow withdrawing the commonly used hyper-hydration following HDMTX infusion, and therefore provide optimal peak and AUC; iii) this simplified, patient-friendly method of administration could facilitate nursing care and allow the outpatient administration of HDMTX.

The present study aimed to demonstrate the reliability and renal safety of a new schedule of HDMTX administration with hyper-alkalinization, and without hyper-hydration.

Patients and methods

From 2001 to 2007, 26 patients with newly diagnosed, high grade OS were treated in our institution using a specific hyper-alkalinization protocol for HDMTX administration, as described below. The first ten patients were treated in the inpatient setting. Given the good tolerability of this regimen, the 16 following patients were treated in the outpatient setting.

The study was approved by the local ethics committee, and informed consent was obtained from each patient or guardian. The medical records of each patient were prospectively entered in a computed database. Patients had received no previous anticancer therapy, and had a normal baseline renal function. They had no concomitant disease potentially leading to acute renal dysfunction.

Treatment protocol

Patients received pre-operative chemotherapy for 8 weeks, followed by 2 weeks off treatment before radical excision surgery was performed. HDMTX was given as a single agent (8-12 g/m²) on day 1, then on days 8 and 15. In addition, patients received ifosfamide (5 g/m², 4 courses), cisplatin (100 mg/m², 1 course) and doxorubicin (60 mg/m², 2 courses) given sequentially, as previously described [16-18]. Anti-emetic prophylaxis included anti-5HT₃ and corticosteroids at standard doses. From 2006, anti-NK1 (aprepitant) was added to this regimen. Within 6 weeks after surgery, patients received adjuvant chemotherapy based on the same regimen in good responders to pre-operative chemotherapy.

Administration of HDMTX

Patients received 500 ml of 8.4% sodium bicarbonate (500 mEq, B Braun Medical, Boulogne-Billancourt, France) over 1 h before HDMTX infusion (day 1), then on days 2, 3 and 4. From day 1 to day 4, patients also received 4 g (53.6 mEq) of KCl daily IV supplementation. The dose of HDMTX was 8 g/m² (4 patients) or 12 g/m² (22 patients). HDMTX dose was diluted in 500 ml of 0.9% NaCl and infused over a 4 h period. HDMTX was not followed by forced hydration, and the IV route was stopped for the following 20 hours. Leucovorin rescue was introduced 24 hr after start of HDMTX infusion (20 hr after completion of the 4 hour infusion) [6]. Dose of folinic acid was subsequently adjusted as a function of serum

methotrexate levels, as previously described (Table 2) [12,15]. On day 4, leucovorin was discontinued and patients were discharged to home once serum methotrexate levels reached ≤ 0.1 mM/L. Patients received the instruction to maintain oral hydration >2 L per 24 h for 3 consecutive days upon discharge.

Sample and pharmacokinetics analysis

Urinary pH was measured on each miction for 3 days following HDMTX infusion. Serum creatinine was measured on days 1, 3 and 8. Methotrexatemia was measured at H0, H4 (C_{\max}), H24, H48 and H72. HDMTX pharmacokinetic parameters were estimated using the software package Thermo Kinetica 4.4 (Inna Phase Corp., USA) and assuming a non-compartmental analysis (NCA).

Analysis of toxicity

Creatinine clearance was calculated according to the Cockcroft and Gault formula [24]. The degree of nephrotoxicity and other adverse events were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) version 2.0. In addition to short-term toxicities, we investigated whether this method of administration of HDMTX induced progressive renal dysfunction, defined as a significant increase of serum creatinine or decrease in calculated creatinine clearance, from baseline to the end of treatment.

Statistical analysis

Student's t test was used to analyse changes of serum creatinine and calculated creatinine clearance from baseline to the end of treatment. Calculations were performed with NCSS 2007 software (NCSS, Kaysville, UT).

Results

Patients characteristics are summarized in Table 3. Amongst the 26 patients (9 females, 17 males), the primary tumour site was axial in 4 patients (15%), and limbs in 22 patients (85%). Three patients (11.5%) had synchronous lung metastases. The median age was 18 years (range: 15-25). A total of 344 cycles, including the pre-operative and post-operative treatments, were evaluable for toxicity and for HDMTX pharmacokinetics (Table 4).

Renal safety of HDMTX given with hyper-alkalinisation and without hyper-hydration

Urinary pH remained constantly higher than 7.5 in all patients. In 31 cycles (9%) of HDMTX, reversible creatinemia elevation (grade 1, < 1.5 ULN) was noticed. Grade 2 creatinine elevation was observed in one patient. No episode of acute severe nephrotoxicity was observed. Regarding progressive renal dysfunction, no significant worsening was observed in serum creatinine and calculated creatinine clearance from baseline to the end of therapy ($p = 0.74$) (Table 1). **No patient required the administration of carboxypeptidase.**

Extra-renal toxicity

Toxicity included manageable grade 1-2 nausea and emesis in all patients, and grade 2 transaminase elevation in 42% of patients. Grade 3 neutropenia occurred in 65 % of patients, and febrile neutropenia in 1 patient. Grade 1-2 mucositis occurred in 26% of patients.

The main acute toxicity was grade 3 hypokaliemia, which occurred in 5 patients. Because hypokaliemia was not associated with serum creatinine elevation, it was supposed to be

secondary to hyper-alkalinization. Hence, hypokaliemia was easily managed with IV or oral KCl supplementation from H24. No grade 4 toxicity occurred.

Although the activity of this regimen was not the endpoint of the study, we observed pathological tumor necrosis rates following pre-operative chemotherapy within the expected range with HDMTX-based regimens [7-10] (median: 95%, range: 15-100%).

Discussion

In the previously described chemotherapy protocols in OS patients, HDMTX was always administered sequentially with other active drugs (cisplatin and ifosfamide) because of its threatening nephrotoxicity. Such neoadjuvant protocols were scheduled over more than 10 weeks, thus lowering chemotherapy dose-intensity, in spite of the theoretical knowledge that dose-intensive treatments warrant a better efficacy in chemosensitive tumours [8,20]. Hence, the optimal neoadjuvant chemotherapy regimen in OS remains to define, and better ways to increase dose intensity with acceptable toxicity could possibly improve results.

The aim of this study was to optimize HDMTX administration by avoiding its nephrotoxicity with aggressive hyper-alkalinization, and to assess the impact of the lack of forced hydration on the pharmacokinetics of HDMTX. There was an obvious intra-patient and inter-patient variability in HDMTX serum concentrations, as previously described in the literature [21,22]. We demonstrated that the administration of HDMTX with hyper-alkalinization and without forced hydration is a safe and reliable method to prevent nephrotoxicity: over 344 cycles, no acute renal dysfunction was noticed. Urinary pH remained stable and > 7.5 , avoiding precipitation of MTX and its metabolites in the renal tubules. **In previous studies, the overall**

incidence of significant acute nephrotoxicity was close to 1.8% [14], whereas another case series reported 30% of patients had a doubling of the baseline serum creatinine [12]. Acute nephrotoxicity was seen in 4% of patients in the present case series of 344 cycles, a proportion that favourably compares with previously published data.

Besides, the lack of forced hydration did not significantly altered HDMTX pharmacokinetics. The threshold peak (700-1000 $\mu\text{mol/l}$) [9] recommended to ensure the efficacy of HDMTX in OS were easily reached.

Outpatient administration requires following for safety: i) alkalization (e.g. method reported in this paper, sodium bicarbonate in daily IV fluids), ii) reliable patients (able to return for bag changes and laboratory exams), iii) facility and staff willing to see patients for laboratory exams, IV fluid questions and that troubleshoot clearance problems on a daily basis.

Finally, the renal safety provided by this method allows the administration of HDMTX in the outpatient setting, which became a standard in our institution following the completion of this study.

Beyond the renal safety provided by this method, we suggest that it might also allow the concomitant administration of HDMTX and other active drugs, for instance ifosfamide. Indeed, increasing the dose-intensity of neoadjuvant chemotherapy is a coherent approach in an effort to increase the proportion of patients with a high degree of necrosis in the primary tumour at the time of definitive surgery [7]. The degree of necrosis in the primary tumour predicts subsequent event-free survival (EFS) [23]. Previously published studies established the efficacy of neoadjuvant chemotherapy [24,25], but never demonstrated the superiority of

intensified neoadjuvant chemotherapy. For instance, a recent phase III study [26] compared two chemotherapy regimens associating doxorubicin and cisplatin, using two different dose-intensities. The dose-intensive regimen was associated with an improved rate of good (> 90% necrosis) histologic responses: 36% vs. 50% ($p = 0.003$). Oddly enough, this improvement in histologic response rate did not result in improved progression-free survival or overall survival. To date, no randomized trial assessed the potential benefit of increasing the dose-intensity of HDMTX and ifosfamide. We assume that our new schedule of administration of HDMTX could be a helpful tool for further studies aiming to evaluate the efficacy of a dose-intensive regimen based on the association of HDMTX and ifosfamide in OS patients.

Conclusions

We demonstrated that hyper-alkalinization is an efficient and reliable method to prevent the acute renal toxicity of HDMTX in OS patients. Moreover, this method allows the **safe** administration of HDMTX in the outpatient setting. Further studies will determine whether this method allows the concomitant administration of HDMTX and other active drugs, which might provide a way to increase the dose-intensity of each active agent by increasing dose-density.

Acknowledgements

The authors wish to thank the clinical research staff and nurses who participated to this study.

References

- 1 Marcove RC, Mike V, Hajek JV, Levin AG, Hutter RV. Osteogenic sarcoma under the age of twenty-one. A review of one hundred and forty-five operative cases. *J Bone Joint Surg Am* 1970;52:411–23.
- 2 Rosen G, Marcove RC, Caparros B, Nirenberg A, Kosloff C, Huvos AG. Primary osteogenic sarcoma: the rationale for preoperative chemotherapy and delayed surgery. *Cancer* 1979;43:2163–77.
- 3 Mir O, Ropert S, Goldwasser F. Neoadjuvant chemotherapy with high-dose methotrexate in osteosarcoma. *Lancet Oncol* 2008;9:1198.
- 4 Meyers PA, Heller G, Healey J, Huvos A, Lane J, Marcove R, Applewhite A, Vlamis V, Rosen G. Chemotherapy for nonmetastatic osteogenic sarcoma: the Memorial Sloan-Kettering experience. *J Clin Oncol* 1992;10:5–15.
- 5 Provisor AJ, Ettinger LJ, Nachman JB, Krailo MD, Makley JT, Yunis EJ, Huvos AG, Betcher DL, Baum ES, Kisker CT, Miser JS. Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children's Cancer Group. *J Clin Oncol* 1997;15:76–84.
- 6 Treon SP, Chabner BA. Concepts in use of high-dose methotrexate therapy. *Clinical Chemistry* 1996;42:1322–29.

7 Bacci G, Ferrari S, Picci P, Zolezzi C, Gherlinzoni F, Iantorno D, Cazzola A. Methotrexate serum concentration and histological response to multiagent primary chemotherapy for osteosarcoma of the limbs. *J Chemother* 1996;8:472–8.

8 Bacci G, Picci P, Avella M, Dallari D, Ferrari S, Prasad R, Di Scioscio M, Malaguti C, Caldora P. The importance of dose-intensity in neoadjuvant chemotherapy of osteosarcoma: a retrospective analysis of high-dose methotrexate, cisplatinum and adriamycin used preoperatively. *J Chemother* 1990;2:127–35.

9 Aquerreta I, Aldaz A, Giraldez J, Sierrasesumaga L. Methotrexate pharmacokinetics and survival in osteosarcoma. *Pediatr Blood Cancer* 2004;42:52–8.

10 Graf N, Winkler K, Betlemovic M, Fuchs N, Bode U. Methotrexate pharmacokinetics and prognosis in osteosarcoma. *J Clin Oncol* 1994;12:1443–51.

11 Ferrari S, Orlandi M, Avella M, Caldora P, Ferraro A, Ravazzolo G, Bacci G. Effects of hydration on plasma concentrations of methotrexate in patients with osteosarcoma treated with high doses of methotrexate. *Minerva Med* 1992;83:289–93.

12 Green MR, Chamberlain MC. Renal dysfunction during and after high-dose methotrexate. *Cancer Chemother Pharmacol* 2009;63:599–604.

13 Sand TE, Jacobsen S. Effect of urine pH and flow on renal clearance of methotrexate. *Eur J Clin Pharmacol* 1981;19:453–6.

14 Widemann BC, Balis FM, Kempf-Bielack B, Bielack S, Pratt CB, Ferrari S, et al. High-dose methotrexate-induced nephrotoxicity in patients with osteosarcoma. *Cancer* 2004;100:2222–32.

15 Messman RA, Allegra CJ: Antifolates; in Chabner BA, Longo DL (eds): *Cancer chemotherapy and biotherapy*. Philadelphia, Lippincott Williams & Wilkins, 2001, pp 139–84.

16 Winkler K, Beron G, Delling G, Heise U, Kabisch H, Purfürst C, Berger J, Ritter J, Jürgens H, Gerein V, et al. Neoadjuvant chemotherapy of osteosarcoma: results of a randomized cooperative trial (COSS-82) with salvage chemotherapy based on histological tumor response. *J Clin Oncol* 1988;6:329–37.

17 Fuchs N, Bielack SS, Epler D, Bieling P, Delling G, Körholz D, Graf N, Heise U, Jürgens H, Kotz R, Salzer-Kuntschik M, Weinel P, Werner M, Winkler K. Long-term results of the co-operative German-Austrian-Swiss osteosarcoma study group's protocol COSS-86 of intensive multidrug chemotherapy and surgery for osteosarcoma of the limbs. *Ann Oncol* 1998;9:893–9.

18 Bacci G, Briccoli A, Ferrari S, Longhi A, Mercuri M, Capanna R, Donati D, Lari S, Forni C, DePaolis M. Neoadjuvant chemotherapy for osteosarcoma of the extremity: long-term results of the Rizzoli's 4th protocol. *Eur J Cancer* 2001;37:2030–9.

19 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16:31–41.

- 20 Norton L, Simon R. Tumor size, sensitivity to therapy, and design of treatment schedules. *Cancer Treat Rep* 1977;61:1307–17.
- 21 Ferrari S, Sassoli V, Orlandi M, Strazzari S, Puggioli C, Battistini A, Bacci G. Serum methotrexate (MTX) concentrations and prognosis in patients with osteosarcoma of the extremities treated with a multidrug neoadjuvant regimen. *J Chemother* 1993;5:135–41.
- 22 Comandone A, Passera R, Boglione A, Tagini V, Ferrari S, Cattel L. High dose methotrexate in adult patients with osteosarcoma: clinical and pharmacokinetic results. *Acta Oncol* 2005;44:406–11.
- 23 Bacci G, Mercuri M, Longhi A, Ferrari S, Bertoni F, Versari M, Picci P. Grade of chemotherapy-induced necrosis as a predictor of local and systemic control in 881 patients with non-metastatic osteosarcoma of the extremities treated with neoadjuvant chemotherapy in a single institution. *Eur J Cancer* 2005; 41: 2079-85.
- 24 Bacci G, Ferrari S, Longhi A, Forni C, Giacomini S, Lari S, Versari M. Relationship between dose-intensity of treatment and outcome for patients with osteosarcoma of the extremity treated with neoadjuvant chemotherapy. *Oncol Rep* 2001;8:883–8.
- 25 Meyers PA, Gorlick R, Heller G, Casper E, Lane J, Huvos AG, Healey JH. Intensification of preoperative chemotherapy for osteogenic sarcoma: results of the Memorial Sloan-Kettering (T12) protocol. *J Clin Oncol* 1998;16:2452–8.

26 Lewis IJ, Nooij MA, Whelan J, Sydes MR, Grimer R, Hogendoorn PC, Memon MA, Weeden S, Uscinska BM, van Glabbeke M, Kirkpatrick A, Hauben EI, Craft AW, Taminiou AH; MRC BO06 and EORTC 80931 collaborators; European Osteosarcoma Intergroup. Improvement of histologic response but not survival in osteosarcoma patients treated with intensified chemotherapy: a randomised phase III trial of the European Osteosarcoma Intergroup. *J Natl Cancer Inst* 2007;99:112–28.

Table 1: Solubility of MTX and its metabolites (mg/ml) [15]

pH	MTX	7-OH MTX	DAMPA
5.0	0.39	0.13	0.05
6.0	1.55	0.37	0.10
7.0	9.04	1.55	0.85

Abbreviations: MTX, methotrexate; 7-OH MTX, 7-hydroxy methotrexate; DAMPA, 2,4-diamino-N₁₀-methyl pteric acid.

Table 2: Leucovorin rescue according to methotrexate levels 48 hours after the start of HDMTX infusion [15]

MTX concentration	Leucovorin dose
5×10^{-7} mol/l	15 mg/m ² q6h x 8
1×10^{-6} mol/l	100 mg/m ² q6h x 8
2×10^{-6} mol/l	200 mg/m ² q6hx8

Abbreviations: MTX, methotrexate.

Table 3: Patients characteristics (n = 26)

Characteristic	Results
Median age (range)	18 (15-25)
Gender: n (%)	
- male	17 (65)
- female	9 (35)
Primary site: n (%)	
- Axial	4 (15)
- Limbs	22 (85)
Median no. of cycles received (range)	11 (2-16)
Baseline Scr ($\mu\text{mol/l}$), mean (\pm SD)	68.5 (\pm 21.5)
EOT SCr ($\mu\text{mol/l}$), mean (\pm SD)	69.7 (\pm 17.5)
Baseline CrCl (ml/min), median (range)	88 (72-159)
EOT CrCl (ml/min), median (range)	92 (70-152)

Abbreviations: EOT, end of treatment; SCr, serum creatinine; CrCl, creatinine clearance, SD: standard deviation.

Table 4: HDMTX pharmacokinetic parameters (n=26)

Dose	n	Mean C_{max} ($\mu\text{mol/l}$)	Mean C_{H24} ($\mu\text{mol/l}$)	Mean C_{H48} ($\mu\text{mol/l}$)	Median AUC ($\mu\text{mol/l.h}$)
HDMTX 8 g/m ²	4	1088 (\pm 277.4)	12.57 (\pm 2.14)	0.48 (\pm 0.31)	4414 (3317-4955)
HDMTX 12 g/m ²	22	1266.9 (\pm 295.8)	25.48 (\pm 1.67)	1.82 (\pm 0.25)	5217 (3966-6118)