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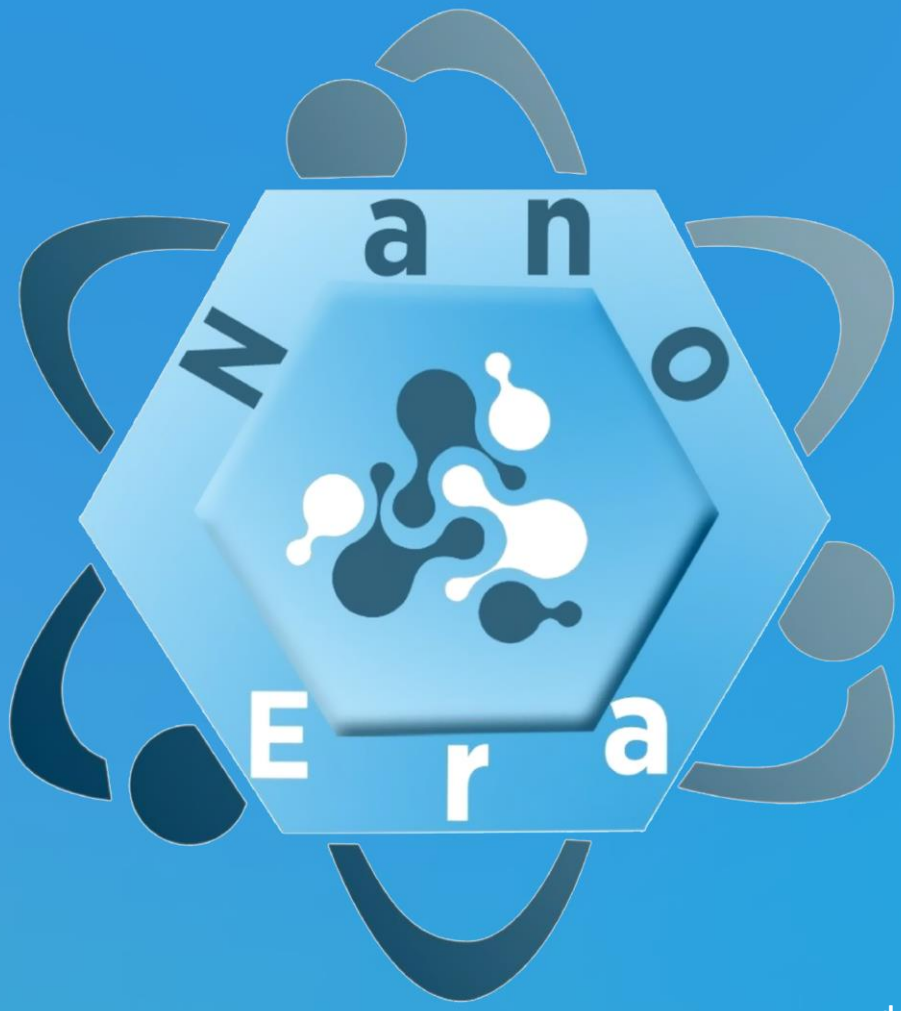


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# NanoEra



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# NanoEra

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ATATÜRK ÜNİVERSİTESİ YAYINLARI
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# Determination of Naproxen using derivatization with MSTFA in pharmaceutical preparations by GC-MS method

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## ABSTRACT

This work reports the using of N-methyl-N-(trimethylsilyl) trifluoroacetamide for the derivatization of naproxen and the GC-MS method for naproxen detection in pharmaceutical preparations. The linearity of the GC-MS technique was proven over the concentration range of 0.5-12 µg/mL. There was less than 1.20 and 1.73% for the intra- and inter-day relative standard deviation, respectively. For the GC-MS technique, the limits of detection and quantification were established at 0.05 and 0.15 µg/mL. The developed technique was used to analysis tablets of a pharmaceutical preparation called Aleve. At the chosen assay conditions, tablet excipients did not cause any interference.

**Keywords:** Naproxen, GC-MS, Derivatization, Validation

## INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen (6-methoxy-a-methyl-2-naphthalene acetic acid) are commonly used to treat a variety of illnesses by providing mild to moderate pain relief.[1] NSAIDs, such as naproxen, are frequently used to treat persistent inflammation, pain, and fever because they can inhibit the cyclooxygenase (COX) enzymes, COX-1 and COX-2, which both produce prostaglandins. These compounds serve a number of vital purposes, including promoting inflammation, pain, and fever.[2] On the other hand, prostaglandins generated by the COX-1 enzyme can also promote blood coagulation, platelet support, and stomach protection. Therefore, NSAIDs have the potential to induce stomach ulcers and increase bleeding following surgery or trauma. Additionally, they are linked to a range of less serious side effects including nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headaches, and sleepiness, as well as potentially major adverse effects like kidney failure.[3]

Patients are exposed to anti-inflammatory medications for extended periods of time when they receive chronic treatment, like in the case of rheumatoid arthritis. Potential abuse and unintentional consumption of naproxen residues in food may put people at risk for health problems like nephrotoxicity, severe gastrointestinal lesions, allergies, and changes in renal function.[4]

Naproxen has been determined using a variety of techniques in plasma, serum, and urine. These techniques include high performance liquid chromatography (HPLC) using UV[5-11] or fluorescence detection,[12-16] liquid chromatography-tandem mass spectrometry (LC-MS/MS),[17,18] gas chromatography-mass spectrometry (GC-MS),[19,20] and gas chromatography-flame ionization detection (GC-FID).[21-23]

In the event that the laboratory does not have access to the most recent, costly technique, GC-MS is now an acceptable substitute for LC-MS/MS. High-resolution capillary GC-MS has not been employed as frequently as LC-MS/MS.[24-28]

Using LC-MS in full-scan MS mode, Sultan et al.[17] and Miksa et al.[18] presented two techniques to simultaneously measure and identify naproxen and other NSAIDs in human plasma. Though their run times were roughly 15 minutes and their LOQ values were relatively high at 2 µg/mL and 20 µg/mL, respectively, both techniques were sturdy and dependable.

There is currently no method available in the literature for determining naproxen by GC-MS in pharmaceutical formulations. Consequently, the O-silylation of naproxen's hydroxyl group is described in this work utilizing N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) as the silylating reagent. Linearity, stability, precision, accuracy, recovery, and sensitivity criteria were used to validate the devised approach in accordance with International Conference on Harmonization (ICH) guidelines.[23] Additionally, it is offered here the naproxen determination using GC-MS technique in pharmaceutical compositions. As a pharmaceutical preparation, Aleve tablets can be prepared with ease using the accurate, sensitive, and exact procedure suggested in this study.

## METHODS

### Chemicals

Sigma (St. Louis, MO, USA) provided the acetonitrile, naproxen sodium, and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). We purchased one Aleve tablet (220 mg naproxen sodium) from the pharmacy in Erzurum, Turkey.

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**Instrumentation**

An Agilent 6890N gas chromatography system (Agilent Technologies, Palo Alto, CA) outfitted with a 5973 series mass selective detector, 7673 series autosampler, and Agilent chemstation was used to perform the chromatographic study. For separation, an HP-5 MS column (30 m × 0.25 mm I.D., USA) with a film thickness of 0.25 µm was employed. Helium was employed as the carrier gas, and splitless injection was performed, with a flow rate of 1 mL/min. One µL was the injector volume. The MS detector's settings included an electron energy of 70 eV, a solvent delay of two minutes, and a transfer line temperature of 280 °C.

**Preparation of the standard and quality control solutions**

Naproxen stock solution was made with acetonitrile at a concentration of 100 µg/mL and refrigerated at -20 °C. For GC-MS, standard solutions of 0.5-12 µg/mL were produced. Aliquots of the standard working naproxen solution were added to the quality control (QC) solutions until the final concentrations for GC-MS were 3, 5, and 9 µg/mL.

**Procedure for pharmaceutical preparations**

The mass of the Aleve (220 mg naproxen tablet, which contained naproxen and a few excipients) capsules was used to compute the average capsule mass. Following its fine grinding and homogenization, a quantity of the powder was precisely weighed, put into a 10 mL brown measuring flask, and diluted with acetonitrile to the appropriate concentration. To speed up the dissolving process, the mixture was sonicated for at least ten minutes before being filtered through a Whatman 42 paper. To ensure that the final solution's naproxen concentration was within the working range, a suitable volume of filtrate was further diluted with acetonitrile before being subjected to GC-MS analysis.

## RESULTS

**Method development and optimization**

Since naproxen is a polar molecule, separation was achieved using a capillary column covered with 95% dimethylpolysiloxane and 5% phenyl. The temperatures of the injection port and detector were set at 250 °C and 290 °C, respectively, during the development of the GC-MS technique. In order to determine the ideal temperature program, several temperature programs were examined. The first temperature was 150 °C, which was held for one minute; it was then raised to 180 °C at 40 °C/min and maintained for another minute; ultimately, it was raised to 300 °C at 30 °C/min with a final hold of 1.5 minutes. In split-less mode, the injector volume was one microliter.

An efficient trimethylsilyl (TMS) donor is MSTFA. MSTFA is used to prepare volatile and thermally stable derivatives for GC-MS by reacting with a TMS group to substitute labile hydrogens on a variety of polar molecules.[24,25] Naproxen was derivatized using MSTFA in order to improve the performance of the gas chromatographic separation (Fig. 1). The appropriate silyl (-O-TMS) groups were formed by converting the hydroxy (-OH) groups. The chemical was examined after the ideal reaction conditions were determined.

Investigations were conducted into how temperature and time affected the reaction. Consequently, acetonitrile was used to dissolve naproxen. After adding 100 µL of 2.0 µg/mL naproxen solution and 100 µL of MSTFA solution, the mixture was allowed to react for five, ten, and twenty minutes at room temperature, 50, and 75 °C. The GC-MS technique was used to quantify the final samples. Following a 10-minute standing period at room temperature, the maximum peak areas were measured.

**Validation of the method**

**Specificity**

Interferences between naproxen and the excipients were observed to examine the specificity of the two approaches. For the quantitative measurement in GC-MS, electron impact mode with selected ion monitoring (SIM) was utilized (m/z 185 for naproxen-TMS). Fig. 1 displays the mass spectrum following the derivatization of naproxen with MSTFA. Naproxen-TMS retention time for GC-MS was around 6.4 with a well-defined peak (Fig. 2).

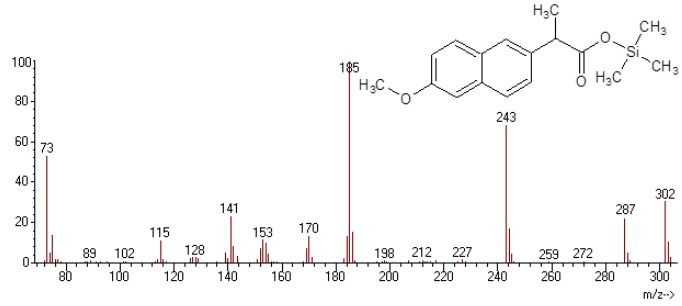


**Fig. 1.** MS spectrum of naproxen-TMS.

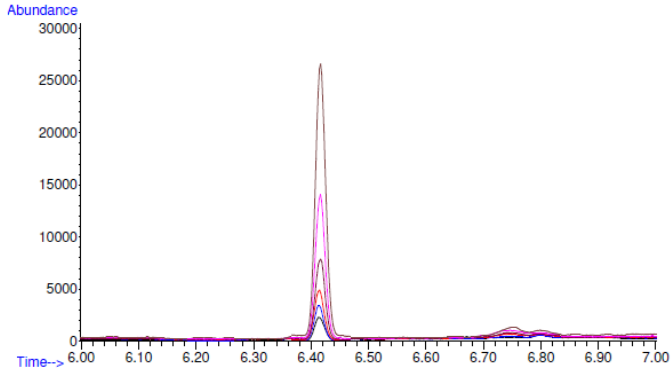


**Fig. 2.** GC-MS chromatogram of naproxen-TMS (0.5, 1, 2, 4, 8 and 12 µg/mL).

**Linearity**

The GC-MS technique revealed that the linearity range of naproxen solution was 0.5-12 µg/mL. Their correlation coefficient was used to assess the calibration curve that was built. The linearity of the procedure was shown by the calibration equation derived from six repeat trials. Table 1 provided the slope and intercept standard deviations for the calibration curves.

**Table 1.** Linearity of naproxen

| Parameter | GC-MS |
|---|---|
| Linearity (µg/mL) | 0.5-12 |
| Regression equation[a] | y=174.4x+5.19 |
| Standard deviation of slope | $2.58x10^{-3}$ |
| Standard deviation of intercept | 3.42 |
| Correlation coefficient | 0.9991 |
| Standard deviation of correlation coefficient | $1.25x10^{-3}$ |
| Limit of detection (µg/mL) | 0.05 |
| Limit of quantification (µg/mL) | 0.15 |

[a]Based on six calibration curves, y=peak area, x=concentration of naproxen

**Accuracy and precision**

Repeatability (intra-day) and intermediate precision (inter-day) were used to calculate the GC-MS method's precision.[25] The study assessed repeatability by subjecting quality control samples to six daily analyses at three distinct doses. The same samples were analyzed twice a day for three days to assess the intermediate precision. The % relative error was used to evaluate the analytical method's accuracy. Relative errors were ≤4.00 % and intra- and inter-day RSD values were ≤1.73% for all concentrations examined. Table 2 presented the findings.

**Limits of detection (LOD) and quantification (LOQ)**

The lowest concentration of the analyte that the technique can detect is known as the LOD. The lowest measurable concentration is known as the LOQ. LOD and LOQ were determined by taking the signal-to-noise ratios of 3:1 and 10:1, respectively. For GC-MS, the LOD and LOQ were 0.05 and 0.15 µg/mL, respectively.

**Stability**

Standard solutions were made independently at concentrations spanning the low, middle, and higher ranges of calibration curves for various temperatures and timeframes to assess the stability of naproxen. For 24 and 48 hours, these solutions were kept at ambient temperature, chilled (4 °C), and frozen (-20 °C). For room temperature, 4 and 20 °C refrigeration temperatures, the accuracy of naproxen stability was found to be 99.8, 98.5, and 99.5%, respectively. These fall between 90 and 110 percent of what is acceptable. Table 3 presented the findings.

**Recovery**

Studies on recovery achieved by spiking various amounts of pure drug within the analytical concentration range of the suggested approach in tablet samples that had already been preanalyzed. Using the aforesaid procedure, the additional dosages of each medicine were estimated. The findings of the recovery experiments were deemed adequate and are displayed in Table 4.

**Table 2.** Precision and accuracy of naproxen

| | Intra-day | | | Inter-day | | |
|---|---|---|---|---|---|---|
| Added (µg/mL) | Found ± SD[a] (µg/mL) | Precision % RSD[b] | Accuracy[c] | Found ± SD[a] (µg/mL) | Precision % RSD[b] | Accuracy[c] |
| 3 | 3.08 ± 0.037 | 1.20 | 2.67 | 3.12 ± 0.054 | 1.73 | 4.00 |
| 5 | 5.02 ± 0.069 | 1.37 | 0.40 | 4.98 ± 0.034 | 0.68 | -0.40 |
| 9 | 9.03 ± 0.143 | 1.58 | 0.33 | 9.01 ± 0.034 | 0.38 | 0.11 |

SD[a]: Standard deviation of six replicate determinations, RSD[b]: Relative standard deviation, Accuracy[c]: % relative error: (found-added)/addedx100

**Table 3.** Stability of naproxen in solution (n=6)

| | Intra-day | | | Inter-day | |
|---|---|---|---|---|---|
| Conc. (µg/mL) | Room temperature 24 h | Refrigeratory 4 °C, 24 h | Frozen -20 °C, 24 h | Refrigeratory 4 °C, 48 h | Frozen -20 °C, 48 h |
| 0.5 | 99.4 ± 3.12 | 97.7 ± 4.97 | 99.6 ± 3.78 | 99.3 ± 2.16 | 99.1 ± 3.26 |
| 6 | 99.7 ± 2.08 | 98.6 ± 3.06 | 100.7 ± 3.15 | 99.5 ± 1.41 | 99.8 ± 2.19 |
| 12 | 100.6 ± 1.89 | 102.1 ± 3.54 | 101.8 ± 2.76 | 100.4 ± 2.56 | 100.6 ± 2.39 |

**Table 4.** Recovery of naproxen in pharmaceutical preparation

| | | Intra-day | | Inter-day | |
|---|---|---|---|---|---|
| **Pharmaceutical preparation** | **Added (µg/mL)** | **Found ± SD[a] (µg/mL)** | **% Recovery % RSD[b]** | **Found ± SD[a] (µg/mL)** | **% Recovery % RSD[b]** |
| **Aleve tablet (2 µg/mL)** | 4 | 4.11 ± 0.049 | 102.8 (1.19) | 4.16 ± 0.093 | 104.0 (2.24) |
| | 7 | 7.04 ± 0.079 | 100.6 (1.12) | 7.09 ± 0.155 | 101.3 (2.19) |
| | 10 | 10.17 ± 0.078 | 101.7 (0.77) | 10.22 ± 0.129 | 102.2 (1.26) |

SD[a]: Standard deviation of six replicate determinations, RSD[b]: Relative standard deviation

### Comparison of the methods

Nowadays, as analytical procedures for both quantitative and qualitative analysis, GC-MS and HPLC methods are significant and often utilized. High-resolution capillary GC-MS has superior precision and accuracy with high sensitivity and strong resolving power compared to HPLC.[26]

The sensitivity of the GC-MS technique is insufficient to determine naproxen. MSTFA is utilized to boost sensitivity as a result. The derivatization procedure in this study is intended to increase sensitivity, which has allowed for the option of operating at low concentrations.

This method's sensitivity was determined to be sufficient for pharmacokinetic research when used with plasma samples. Compared to the reported approach, the current method has the following advantages.[17, 18] The current method's limit of quantification (LOQ) was 0.15 µg/mL, while the reported methods' LOQs were 2.0 µg/mL and 20 µg/mL. The naproxen calibration curve was linear over the 0.25-5.0 µg/mL concentration range, which is on par with or better than that found in earlier studies.[5-8,12-15,17,20–22]

This study has a high naproxen recovery rate.[12, 13, 18] Another benefit is the short retention duration.[5, 9, 12, 14, 17, 18, 20]

## DISCUSSION

This paper presents the development and validation of a novel chromatographic method for routine measurement of naproxen in pharmaceutical formulations. Proper linearity range, accuracy, precision, LOD, and LOQ are appropriate for naproxen quantification in pharmaceutical compositions. A significant number of samples can be analyzed quickly because of the 7-minute chromatographic run time. As a result, the technique can also be used to analyze samples during expedited stability investigations as well as regular formulation and raw material analyses.

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**Conflict of Interest:** The authors declare that they have no competing interests.

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### REFERENCES

1. Boynton CS, Dick CF, Mayor GH. NSAIDs: An Overview. *J Clin Pharmacol*. 1988;28(6):512-517. doi:10.1002/j.1552-4604.1988.tb03170.x
2. Sun Y, Zhang Z, Xi Z, Shi Z. Determination of naproxen in human urine by high-performance liquid chromatography with direct electrogenerated chemiluminescence detection. *Talanta*. 2009;79(3):676-680. doi:10.1016/j.talanta.2009.04.048
3. Aresta A, Palmisano F, Zambonin CG. Determination of naproxen in human urine by solid-phase microextraction coupled to liquid chromatography. *J Pharm Biomed Anal.* 2005;39(3-4):643-647. doi:10.1016/j.jpba.2005.04.017
4. Kovacevic L, Bernstein J, Valentini RP, Imam A, Gupta N, Mattoo TK. Renal papillary necrosis induced by naproxen. *Pediatr Nephrol*. 2003;18(8):826-829. doi:10.1007/s00467-003-1167-4
5. Van Loenhout JWA, Van Ginneken C a. M, Ketelaars HCJ, Kimenai PM, Tan Y, Gribnau FWJ. A High-Performance Liquid Chromatographic Method for the Quantitative Determination of Naproxen and Des-Methyl-Naproxen in Biological Samples. *J Liq Chromatogr*. 1982;5(3):549-561. doi:10.1080/01483918208066913
6. Zakeri-Milani P, Barzegar-Jalali M, Tajerzadeh H, Azarmi Y, Valizadeh H. Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation. *J Pharm Biomed Anal.* 2005;39(3-4):624-630. doi:10.1016/j.jpba.2005.04.008
7. Slattery JT, Levy G. Determination of naproxen and its desmethyl metabolite in human plasma or serum by high performance liquid chromatography. *Clin Biochem.* 1979;12(3):100-103. doi:10.1016/s0009-9120(79)80075-2
8. Satterwhite JH, Boudinot FD. High-performance liquid chromatographic determination of ketoprofen and naproxen in rat plasma. *J Chromatogr B*. 1988;431:444-449. doi:10.1016/s0378-4347(00)83116-3
9. Upton RA, Buskin JN, Guentert TW, Williams RL, Riegelman S. Convenient and sensitive high-performance liquid chromatography assay for ketoprofen, naproxen and other allied drugs in plasma or urine. *J Chromatogr A*.

1980;190(1):119-128. doi:10.1016/s0021-9673(00)85518-1

10. Vree TB, Van Den Biggelaar-Martea M, Verwey-van Wissen CPWGM. Determination of naproxen and its metabolite O-desmethylnaproxen with their acyl glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatography. *J Chromatography B*. 1992;578(2):239-249. doi:10.1016/0378-4347(92)80422-m
11. Hirai T, Matsumoto S, Kishi I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatography B*. 1997;692(2):375-388. doi:10.1016/s0378-4347(96)00509-9
12. Shimek JL, Rao NGS, Wahba Khalil K. An isocratic high-pressure liquid chromatographic determination of naproxen and desmethylnaproxen in human plasma. *J Pharm Sci*. 1982;71(4):436-439. doi:10.1002/jps.2600710415
13. Burgoyne RF, Brown PR, Kaplan SR. The simultaneous assay of naproxen and salicylic acid in serum using high pressure liquid chromatography. J Liq Chromatogr. 1980;3(1):101-111. doi:10.1080/01483918008060157
14. Westerlund D, Theodorsen A, Jaksch Y. Bioanalysis of Naproxen by High Performance Reversed Phase Liquid Chromatography with Photometric and Fluorimetric Detection. *J Liq Chromatogr*. 1979;2(7):969-1001. doi:10.1080/01483917908060119
15. Tashtoush, B.M., Al-Taani B.M.; HPLC determination of naproxen in plasma, *Pharmazie,* (2003);58:614-615.
16. Mikami E, Goto T, Ohno T, Matsumoto H, Nishida M. Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphthylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography. *J Pharm Biomed Anal*. 2000;23(5):917-925. doi:10.1016/s0731-7085(00)00365-4
17. Sultan M, Stecher G, Stoggl W, et al. Sample pretreatment and determination of non steroidal anti-inflammatory drugs (NSAIDs) in pharmaceutical formulations and biological samples (blood, plasma, erythrocytes) by HPLC-UV-MS and HPLC. *Curr Med Chem.* 2005;12(5):573-588. doi:10.2174/0929867310504050573
18. Miksa IR, Cummings MR, Poppenga RH. Multi-residue determination of anti-inflammatory analgesics in sera by liquid chromatography-mass spectrometry. *J Anal Toxicol*. 2005;29(2):95-104. doi:10.1093/jat/29.2.95
19. Larsen NE, Marinelli K. Mass fragmentographic quantification of naproxen in human plasma. *J Chromatography B*. 1981;222(3):482-485. doi:10.1016/s0378-4347(00)84151-1
20. Wan SH, Matin SB. Quantitative gas-liquid chromatographic analysis of naproxen, 6-O-desmethyl-naproxen and their conjugates in urine. *J Chromatography A*. 1979;170(2):473-478. doi:10.1016/s0021-9673(00)95480-3
21. Weber SS, Bankhurst AD, Mroszczak E, Ding TL. Effect of mylanta® on naproxen bioavailability. *Ther Drug Monit*. 1981;3(1):75-84. doi:10.1097/00007691-198101000-00010
22. Desager JP, Vanderbist M, Harvengt C. Naproxen plasma levels in volunteers after single-dose administration by oral and rectal routes. *J Clin Pharmacol*. 1976;16(4):189-193. doi:10.1002/j.1552-4604.1976.tb01516.x
23. Bouchafra H, Orche AE, Khabbaz CE, Cheikh A, Faouzi MEA, Cherrah Y, Zarayby L, Hirr A. Determination and validation of tiaprofenic acid in human plasma: A detailed LC-MS/MS-based analysis following ICH M10 guidelines and the accuracy profile approach. *Research Square.* 2024;1-16. doi: https://doi.org/10.21203/rs.3.rs-3848449/v1
24. Yilmaz B, Arslan S, Akba V. Gas chromatography–mass spectrometry method for determination of metoprolol in the patients with hypertension. *Talanta*. 2009;80(1):346-351. doi:10.1016/j.talanta.2009.06.079
25. Yilmaz B. GC–MS determination of diclofenac in human plasma. *Chromatographia*. 2010;71(5-6):549-551. doi:10.1365/s10337-010-1479-z
26. Yilmaz B. Determination of Atenolol in Pharmaceutical Preparations by Gas Chromatography with Flame Ionization and Mass Spectrometric Detection. *Anal Lett*. 2010;43(15):2311-2317. doi: 10.1080/00032711003717414
27. Way BA, Wilhite TR, Smith CH, Landt M. Measurement of plasma ibuprofen by gas chromatography-mass spectrometry. *J Clin Lab Anal*. 1997;11(6):336-339. http://dx.doi.org/10.1002/(sici)1098-2825(1997)11:6<336::aid-jcla4>3.0.co;2-3
28. Sebők Á, Vasanits-Zsigrai A, Palkó Gy, Záray Gy, Molnár-Perl I. Identification and quantification of ibuprofen, naproxen, ketoprofen and diclofenac present in waste-waters, as their trimethylsilyl derivatives, by gas chromatography mass spectrometry. *Talanta*. 2008;76(3):642-650. doi:10.1016/j.talanta.2008.04.008

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# Biotribological behavior of polycaprolactone (PCL)/carbon quantum dots (CQDS) films

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## ABSTRACT

Several new-generation synthetic biodegradable polymers have been developed specifically for biomedical applications in the last two decades. Polycaprolactone (PCL) was chosen as the polymer matrix in this study because it is known for its ease of synthesis, commercial availability, and excellent biocompatibility. Carbon Quantum Dots (CQDs), one of the carbon nanostructures with superior properties, were used as fillers to produce PCL film nanocomposites with improved biotribological properties. The biotribological behavior of (Sample of K-CQDs produced from Rosehip) K-CQDs filled PCL matrix nanocomposite films containing 0.3 and 2.0 wt. % K-CQDs filler were investigated in sliding against an alumina ($Al_2O_3$) counterface by a constant loading (2.5 N) and sliding speed (1.7 cm $s^{-1}$) experiments carried out in a reciprocating friction testing machine in 0.154 M isotonic salt solution. PCL/K-CQDs-2.0 film had lower friction coefficient value (0.304) with a 70% decrease, and wear rate (0.00051 $mm^3$/Nm; 65% decrease) compared to PCL/K-CQDs-0.3. The surface images of PCL/K-CQDs-2.0 film after the wear test indicated that the wear width trace and the adhesive wear traces decreased. In addition, the absence of cracks on the worn surface showed that both films were resistant to plastic deformation.

**Keywords:** Polycaprolactone, Carbon quantum dots, Nanocomposite films, Biotribology

## INTRODUCTION

The application of green technologies has been one of the most studied topics in recent years, improving the properties of biodegradable and biocompatible aliphatic polyesters such as polycaprolactone (PCL) and polylactic acid (PLA).[1] PCL is readily available commercially and has high flexibility. These properties have led to its use in a variety of applications, such as the production of agricultural films, biodegradable food packages, and products used in the healthcare industry, along with the biodegradability and biocompatibility of PCL.[2,3] However, the wide use of PCL has been limited due to its significant disadvantages, such as its low mechanical properties, hardness, strength, and low gas permeability properties. It is necessary to improve the weak properties of PCL while preserving its biodegradable and biocompatible nature.[4] Various methods are used to improve the inadequate properties of PCL in order to increase its use in desired application areas.

Among these methods, nanocomposite production and extrusion with other biodegradable polymers are the most frequently used. Studies are continuing on PCL-based nanocomposites with improved properties by incorporating various types of nanoparticles such as hydroxyapatite, nanoclay, microfibril cellulose, and carbon nanotubes.[5-9] A lot of research has been done on carbon nanostructures because of their superior physicochemical, mechanical, and electrical qualities. These include fullerene, graphene nanosheets, carbon nanotubes (single and multiwalled), carbon nanofibers, and carbon nanoparticles.[10] Since carbon quantum nanodots (CQDs) offer greater qualities over the previously described carbon nanostructures, they have become the subject of numerous studies. CQDs are a brand-new class of zero-dimensional carbon nanomaterials that have special fluorescence qualities and a size of less than 10 nm. Compared to other types of nanomaterials, CQDs have a greater acidity value and surface activity because of the numerous carboxyl and hydroxyl groups that are present on their surfaces. CQDs samples are preferred due to specific chemical or physical interactions between CQDs and polymers.[11] CQDs have been produced by various methods using different carbon sources. Natural carbon sources attract attention because they are abundant, low-cost, and environmentally friendly compared to synthetic carbon sources.[12]

Recently, the use of natural substances in the synthesis of CQDs has become important with the development of green synthesis methods. Green CQDs are some of the many more reported sources, such as banana juice, onions, crab shells, glycerol, and mushrooms.[13] There are different carbon sources used for green synthesized CQDs using the hydrothermal method.[12-22]

In this study, the liquid-phase ultrasonic mixing method was used to produce the biotribological behavior of PCL nanocomposite films by adding K-CQDs samples synthesized from a natural source (Rosehip) using the hydrothermal method.[23] In the literature, the corrosion and wear behaviors of biocompatible $TiO_2$/PCL hybrid layers prepared via sol-gel dip coating on Ti6Al4V implants were

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examined.[24] And, the biodegradability behavior of a uniform PCL coating on a magnesium screw was examined in another study.[25] These studies show that this field is much newer and more remarkable. The friction and wear behavior of PCL/CQDs nanocomposite films have not been studied. This is a very original study on this subject.

## EXPERIMENTAL SECTION

PCL/K-CQDs films were produced according to our previous study.[26] Images of the of PCL/K-CQDs films prepared for the wear test are given in Figure 1. PCL/K-CQDs films were subjected to wear tests using a reciprocating tribometer. The wear tests were conducted with a sliding distance of 50 m and a constant load of 2.5 N at a sliding velocity of 1.7 cm $s^{-1}$. A 2 mm-diameter ball made of $Al_2O_3$ served as the counter body. The friction force was continuously recorded by the computer with the load cell in the wear device. Following the wear test, the Nikon imaging program NIS-Elements was used to measure the 3D profiles and the Mitutoyo Surtest SJ-400 profilometer was used to measure the 2D profiles. An optical metal microscope (OM) Nikon Eclipse LV150 was used to study worn surfaces and surface pictures of $Al_2O_3$ balls. The wear rate was calculated using Equation (1) below. Three samples were used for each experiment.

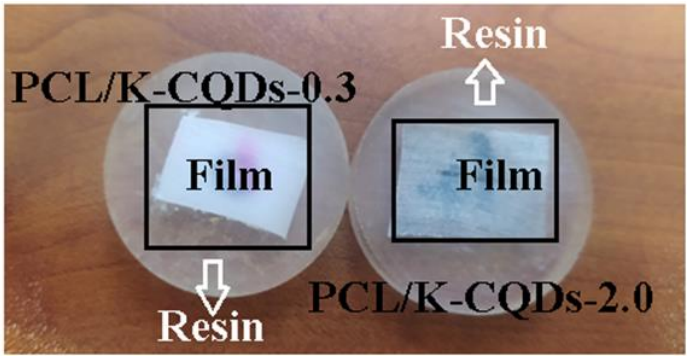


**Fig 1.** Images of the of PCL/CQDs films prepared for the wear test.

$$A = \frac{\pi.W.D.C}{4.S.F} \qquad (1)$$

A: Wear rate, $mm^3/Nm$
W: Width of wear mark, mm
D: Depth of wear mark, mm
C: Length of wear mark, mm
S: Total sliding distance, m
F: Test load, N.

## RESULTS AND DISCUSSION

The variation of the friction coefficient values obtained after the wear tests of PCL/K-CQDs-0.3 and PCL/K-CQDs-2.0 films in 0.154 M NaCl solution according to sliding distance is given in Figure 2. As seen in Figure 2, the friction coefficient values of PCL/K-CQDs-0.3 and PCL/K-CQDs-2.0 films were 1.04 and 0.304, respectively. The oscillation in the friction coefficient of the PCL/K-CQDs-2.0 film was also less than that of the PCL/K-CQDs-0.3 film. Hendrikson *et al.*[27] reported the friction coefficient as 0.529 of the PCL sample for the tissue scaffold at 1N load. In this study, the lower friction coefficient value of the PCL/K-CQDs-2.0 film as a result of the lubricating effect of the liquid medium and the wear test at 2.5 N are compatible with the literature. The friction coefficient values performed in dry environments in the wear tests are higher than the friction coefficient values in liquid environments. Because direct contact is prevented in liquid environments[28] and the friction heat is reduced. And also, it had been reported that the friction coefficient showed a more stable change in the liquid environment.[29,30]

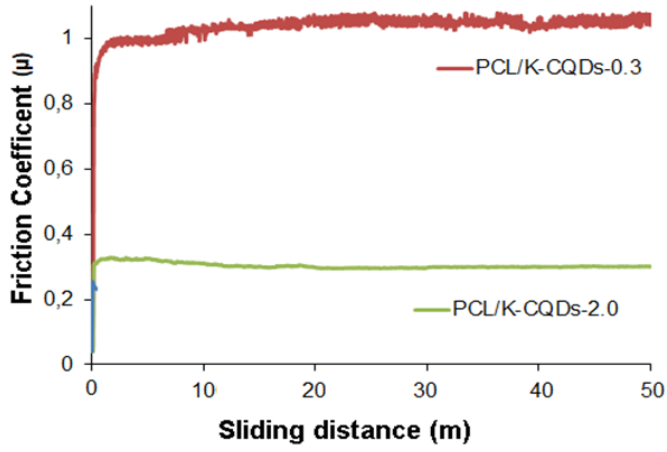


**Fig. 2.** Friction coefficient values of PCL/K-CQDs films.

Table 1 displays the PCL/K-CQDs films' wear volume and wear rate values. The wear volume and wear rate values of the PCL/K-CQDs-0.3 film were higher than the wear results of the PCL/K-CQDs-2.0 film (Table 1). 3D profiles of the wear track of the PCL/K-CQDs films are shown in Figure 3. It can be seen that in the wear track images in Figure 3, the PCL/K-CQDs-0.3 film had the highest wear track. 3D wear track images of the films confirmed the wear rate results. The wear volume of the PCL/K-CQDs-2.0 film was found to be 57% lower, and the wear rate was 59% lower than that of the PCL/K-CQDs-0.3 film.

**Table 1.** The wear volume and wear rate values of the PCL/K-CQDs films

| | Wear volume ($mm^3$) | Wear rate ($mm^3/Nm$) |
|---|---|---|
| PCL/K-CQDs-0.3 | 0.21079 | 0.00147 |
| PCL/K-CQDs-2.0 | 0.07349 | 0.00051 |

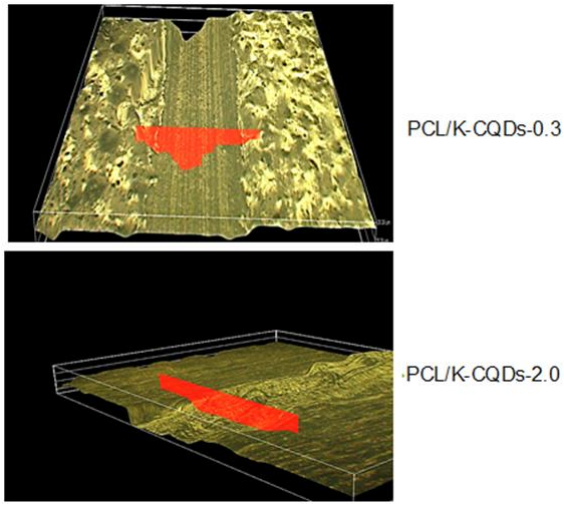


**Fig 3.** 3D profile of wear tracks of PCL/K-CQDs films.

OM worn surface images of PCL/K-CQDs nanocomposite films after the wear test are given in Figure 4. As seen in Figure 4, the PCL/K-CQDs-0.3 film had a higher (829.2 µm) wear track width than that of the PCL/K-CQDs-2.0 film (663.6 µm). High-magnification surface images of the films show that adhesive wear is a dominant mechanism. In a study by Bustillos *et al.*, [31] they produced graphene-reinforced PLA composites. It was reported that the heat generated on the wear surface softened the polymer, causing adhesive wear. This study was carried out in a liquid environment; therefore, frictional heat was largely prevented, and adhesive wear was the dominant mechanism in all films. In addition to adhesive wear, there are traces of large and small pieces broken off from the surfaces of films. It is understood from Figure 4 that, especially in the PCL/K-CQDs-0.3 films, delamination wear was seen in different parts of the surface, and the PCL/K-CQDs-2.0 film had delamination wear in the form of deep grooves Figure 4, the traces that can be seen as cracks on the surfaces in the high-magnification worn surface images were confirmed to be traces of adhesive wear.

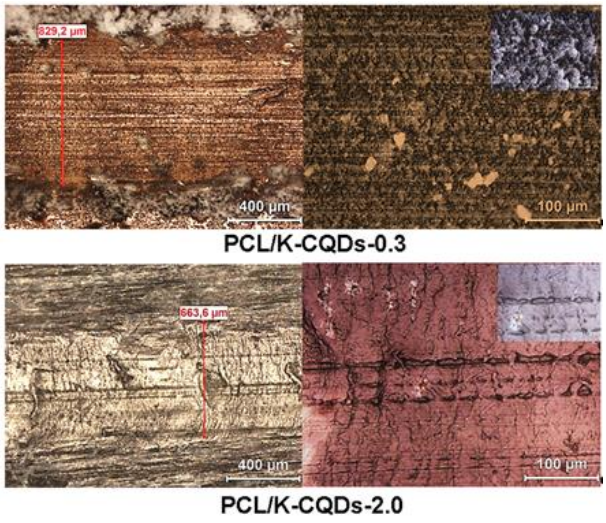


**Fig 4.** Low and high magnification OM images of worn surfaces generated on the PCL/K-CQDs films.

Similar to the results of this study, Min *et al.*,[32] reported that PVA and GO/PVA coatings showed similar wear mechanisms (adhesive) after the wear test, which are biodegradable polymers such as PCL. The same study attributed the observed increase in the friction coefficient to the partial delamination of composite coatings, and cracks do not appear on the surface due to their resistance to plastic deformation. The wear particles did not have an increasing effect on the friction coefficient due to the wear test being carried out in a liquid environment in this study. OM analyses of the surface morphologies of $Al_2O_3$ counterface were evaluated in Figure 5. In Figure 5, it is determined that the wear particles on the ball surface of the PCL/K-CQDs-0.3 film had the highest friction coefficient value. More wear particles were observed on the ball surfaces of the PCL/K-CQDs-2.0 film, which has higher wear resistance than the PCL/K-CQDs-0.3 film. It has been reported in the literature that wear particles form a lubricant film between the ball surface and the worn surface, and this lubricant film contributes to the protection of the composite surface against wear.[33] In this study, the very low presence of wear particles on the counter material of the PCL/K-CQDs-0.3 film explains the high wear volume value of this film However, there is a possibility that the resulting wear particles were removed by the liquid since the wear test was made in a liquid environment in this study. As a result, the addition of 0.3 wt% K-CQDs in this film caused poor wear resistance as it could not provide sufficient load transfer.

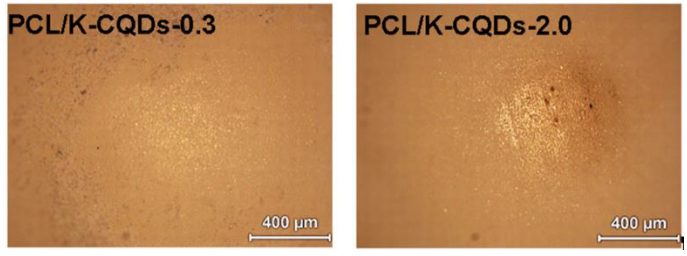


**Fig 5.** OM images of the $Al_2O_3$ balls sliding against the PCL/K-CQDs films.

Wear tests of PCL/K-CQDs-0.3 and PCL/K-CQDs-2.0 films were carried out in a 0.154 M NaCl solution. According to the friction coefficient and wear rate results, the films containing 2.0 wt% K-CQDs showed more resistance to wear. The worn surface images of films demonstrated that adhesive wear accounted for the majority of the wear mechanism, however delamination wear was also noticed. Delamination wear was also seen, however adhesive wear accounted for the majority of the wear mechanism, as shown by the worn surface photographs of the films. However, the absence of cracks on the worn surfaces of both films showed that they were resistant to plastic deformation.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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**REFERENCES**

1. Chen J, Lu L, Wu D, et al. Green Poly(ε-caprolactone) Composites Reinforced with Electrospun Polylactide/Poly(ε-caprolactone) Blend Fiber Mats. *ACS Sustain Chem Eng.* 2014;2(9):2102-2110. doi:10.1021/sc500344n
2. Thakur M, Majid I, Hussain S, Nanda V. Poly(ε-caprolactone): A potential polymer for biodegradable food packaging applications. *Packag Technol Sci.* 2021;34(8):449-461. doi:10.1002/pts.2572
3. Guarino V, Gentile G, Sorrentino L, Ambrosio L. Polycaprolactone: Synthesis, Properties, and Applications. *Encyclopedia of Polymer Science and Technology.* Published online August 15, 2017:1-36. doi:10.1002/0471440264.pst658
4. Woodruff MA, Hutmacher DW. The return of a forgotten polymer—Polycaprolactone in the 21st century. *Prog Polym Sci.* 2010;35(10):1217-1256. doi:10.1016/j.progpolymsci.2010.04.002
5. Baji A, Wong S, Liu T, Li T, Srivatsan TS. Morphological and X-ray diffraction studies of crystalline hydroxyapatite-reinforced polycaprolactone. *J Biomed Mater Res B Appl Biomater*. 2006;81B(2):343-350. doi:10.1002/jbm.b.30671
6. Marras SI, Kladi KP, Tsivintzelis I, Zuburtikudis I, Panayiotou C. Biodegradable polymer nanocomposites: The role of nanoclays on the thermomechanical characteristics and the electrospun fibrous structure. *Acta Biomater.* 2008;4(3):756-765. doi:10.1016/j.actbio.2007.12.005
7. Lönnberg H, Larsson K, Lindström T, Hult A, Malmström E. Synthesis of Polycaprolactone-Grafted Microfibrillated Cellulose for Use in Novel Bionanocomposites–Influence of the Graft Length on the Mechanical Properties. *ACS Appl Mater Interfaces.* 2011;3(5):1426-1433. doi:10.1021/am2001828
8. Villmow T, Kretzschmar B, Pötschke P. Influence of screw configuration, residence time, and specific mechanical energy in twin-screw extrusion of polycaprolactone/multi-walled carbon nanotube composites. *Compos Sci Technol.* 2010;70(14):2045-2055. doi:10.1016/j.compscitech.2010.07.021
9. Kakroodi AR, Kazemi Y, Rodrigue D, Park CB. Facile production of biodegradable PCL/PLA in situ nanofibrillar composites with unprecedented compatibility between the blend components. *Chem Eng J*. 2018;351:976-984. doi:10.1016/j.cej.2018.06.152
10. Mohamed RM, Yusoh K. A Review on the Recent Research of Polycaprolactone (PCL). *Adv Mater Res*. 2015;1134:249-255. doi:10.4028/www.scientific.net/amr.1134.249
11. Caglayan MO, Mindivan F, Şahin S. Sensor and Bioimaging Studies Based on Carbon Quantum Dots: The Green Chemistry Approach. *Crit Rev Anal Chem.* 2020;52(4):814-847. doi:10.1080/10408347.2020.1828029
12. Wongso V, Sambudi NS, Sufian S, Isnaeni N. The effect of hydrothermal conditions on photoluminescence properties of rice husk-derived silica-carbon quantum dots for methylene blue degradation. *Biomass Convers Biorefin.* 2020;11(6):2641-2654. doi:10.1007/s13399-020-00662-9
13. Irmania N, Dehvari K, Gedda G, Tseng P, Chang J. Manganese-doped green tea-derived carbon quantum dots as a targeted dual imaging and photodynamic therapy platform. *J Biomed Mater Res B Appl Biomater*. 2019;108(4):1616-1625. doi:10.1002/jbm.b.34508
14. Yang H, Zhou B, Zhang Y, et al. Valorization of Expired Passion Fruit Shell by Hydrothermal Conversion into Carbon Quantum Dot: Physical and Optical Properties. *Waste Biomass Valor.* 2020;12(4):2109-2117. doi:10.1007/s12649-020-01132-z
15. Arumugham T, Alagumuthu M, Amimodu RG, Munusamy S, Iyer SK. A sustainable synthesis of green carbon quantum dot (CQD) from Catharanthus roseus (white flowering plant) leaves and investigation of its dual fluorescence responsive behavior in multi-ion detection and biological applications. *Sustain Mater Technol.* 2020;23:e00138. doi:10.1016/j.susmat.2019.e00138
16. Tadesse A, Hagos M, RamaDevi D, Basavaiah K, Belachew N. Fluorescent-Nitrogen-Doped Carbon Quantum Dots Derived from Citrus Lemon Juice: Green Synthesis, Mercury(II) Ion Sensing, and Live Cell Imaging. *ACS Omega.* 2020;5(8):3889-3898. doi:10.1021/acsomega.9b03175
17. Chaudhary N, Gupta PK, Eremin S, Solanki PR. One-step green approach to synthesize highly fluorescent carbon quantum dots from banana juice for selective detection of copper ions. *J Environ Chem Eng.* 2020;8(3):103720. doi:10.1016/j.jece.2020.103720
18. Surendran P, Lakshmanan A, Vinitha G, Ramalingam G, Rameshkumar P. Facile preparation of high fluorescent carbon quantum dots from orange waste peels for nonlinear optical applications. *Luminescence.* 2019;35(2):196-202. doi:10.1002/bio.3713
19. Dias C, Vasimalai N, Sárria MP, et al. Biocompatibility and Bioimaging Potential of Fruit-Based Carbon Dots. *Nanomaterials*. 2019;9(2):199. doi:10.3390/nano9020199
20. Rajendran K, Rajendran G, Kasthuri J, Kathiravan K, Rajendiran N. Sweet Corn (Zea mays L. var. rugosa) Derived

Fluorescent Carbon Quantum Dots for Selective Detection of Hydrogen Sulfide and Bioimaging Applications. *ChemistrySelect*. 2019;4(46):13668-13676. doi:10.1002/slct.201903385

**21.** Arkan E, Barati A, Rahmanpanah M, Hosseinzadeh L, Moradi S, Hajialyani M. Green Synthesis of Carbon Dots Derived from Walnut Oil and an Investigation of Their Cytotoxic and Apoptogenic Activities toward Cancer Cells. *Adv Pharm Bull.* 2018;8(1):149-155. doi:10.15171/apb.2018.018

**22.** Vasimalai N, Vilas-Boas V, Gallo J, et al. Green synthesis of fluorescent carbon dots from spices for in vitro imaging and tumour cell growth inhibition. *Beilstein J Nanotechnol.* 2018;9:530-544. doi:10.3762/bjnano.9.51

**23.** Mindivan F, Göktaş M. The green synthesis of carbon quantum dots (CQDs) and characterization of polycaprolactone (PCL/CQDs) films. *Colloids Surf A Physicochem Eng Asp*. 2023;677:132446. doi:10.1016/j.colsurfa.2023.132446

**24.** Catauro M, Bollino F, Giovanardi R, Veronesi P. Modification of Ti6Al4V implant surfaces by biocompatible TiO 2 /PCL hybrid layers prepared via sol-gel dip coating: Structural characterization, mechanical and corrosion behavior. *Mater Sci Eng C Biomim Mater Sens Syst.* 2017;74:501-507. doi:10.1016/j.msec.2016.12.046

**25.** Tong P, Sheng Y, Hou R, Iqbal M, Chen L, Li J. Recent progress on coatings of biomedical magnesium alloy. *Smart Mater Med.* 2022;3:104-116. doi:10.1016/j.smaim.2021.12.007

**26.** Makta S. *Green Synthesis of Carbon Quantum Dots from Rosa Canina L. (K-CQDS), Preparation of K-CQDS Filled Polycaprolacton Films (PCL/K-CQDS), Investigation of Their Mechanical and Biodegradability Properties*. [MSc Thesis]. Bilecik, Turkey; 2023.

**27.** Hendrikson WJ, Zeng X, Rouwkema J, Van Blitterswijk CA, Van Der Heide E, Moroni L. Biological and Tribological Assessment of Poly(Ethylene Oxide Terephthalate)/Poly(Butylene Terephthalate), Polycaprolactone, and Poly (L\DL) Lactic Acid Plotted Scaffolds for Skeletal Tissue Regeneration. *Adv Healthc Mater*. 2015;5(2):232-243. doi:10.1002/adhm.201500067

**28.** Chen B, Wang J, Yan F. Friction and Wear Behaviors of Several Polymers Sliding Against GCr15 and 316 Steel Under the Lubrication of Sea Water. *Tribol Lett.* 2011;42(1):17-25. doi:10.1007/s11249-010-9743-9

**29.** Dangsheng X. Friction and wear properties of UHMWPE composites reinforced with carbon fiber. *Mater Lett.* 2005;59(2-3):175-179. doi:10.1016/j.matlet.2004.09.011

**30.** Meng H, Sui GX, Xie GY, Yang R. Friction and wear behavior of carbon nanotubes reinforced polyamide 6 composites under dry sliding and water lubricated condition. *Compos Sci Technol.* 2009;69(5):606-611. doi:10.1016/j.compscitech.2008.12.004

**31.** Bustillos J, Montero D, Nautiyal P, Loganathan A, Boesl B, Agarwal A. Integration of graphene in poly(lactic) acid by 3D printing to develop creep and wear-resistant hierarchical nanocomposites. *Polym Compos.* 2017;39(11):3877-3888. doi:10.1002/pc.24422

**32.** Min Y, Kim CL, Kim DE. Tribological properties of the hierarchically structured graphene oxide composite coatings reinforced with polyvinyl alcohol. *Wear*. 2022;490-491:204212. doi:10.1016/j.wear.2021.204212

**33.** Jeon H, Kim Y, Yu WR, Lee JU. Exfoliated graphene/thermoplastic elastomer nanocomposites with improved wear properties for 3D printing. *Compos Part B Eng.* 2020;189:107912. doi:10.1016/j.compositesb.2020.107912

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# Experimental evaluation of water source heat pump by Taguchi method

## ABSTRACT

Water source heat pumps are devices that can produce heating or cooling in the place using the energy in the ambient water which serves as the easiest source of heat. The heat pumps, which utilize the ambient water on the boiling side, are able to transfer the energy they have obtained to the water or air on the load side. The renewable geothermal energy, which is produced by the alternative energy sources, is also an important potential in Turkey as a sustainable and environmental- friendly source. The subject of utilizing geothermal energy has been among the most balanced and high efficiency, low maintenance comfortable energy sources in pace with the technological developments of the heat pumps. The widespread and daily use of the ground source heat pumps and the examination of the situation in Turkey lead us to the determination of the potential applicability which is one of the important issues. In this study, the COP of various parameters and different levels of the water source heat pumps were calculated according to the Taguchi experiment design.

**Keywords:** Heat Pumps, COP, Taguchi experiment design, energy

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## INTRODUCTION

The commonly used air source heat pumps are air-to-air heat pumps which are often referred as "air conditioners". Air-to-water heat ramps provide the possibility of more comfortable heating by the floor heating or radiators (static heating) since they can transfer their energy to the load side. In general, cooling by fan coils can be done in the areas where the relative humidity is suitable by using the underfloor heating pipes. The heat pump is a system that simply transfers heat energy from one medium to another while it is powered by the electricity. The heat pump takes its name from its ability to "pump" or "transport" heat energy from one medium to another. So, when the necessary conditions are met, high amounts of energy are offered at low costs.[1]

In general, the heating pumps aim to transport heat than producing it, and they need a heat pit to remove heat. Almost all of the heat pumps used in our country use air as a heat sink. Today, heat pumps using air as a heat sink are called as Split air conditioners. Efficiencies of air-borne devices take different values with changing of outside temperature. The unexpected increases in operating costs occur because the yield values do not remain constant even during the day.[1] There are also heat pits that can accept the temperature as constant while preventing these changes in efficiency. The heat pits which can accept the temperature used for this purpose are soil and water.

Water source heat pump technology at a certain depth of the earth is based on the fact that the temperature remains relatively constant throughout the year. The soil layer in the mentioned depth takes this advantage provided by nature by carrying the heat which is stored under the ground or underground water in the winter to the building, and the heat inside the building underground in the summer. Briefly, underground serves as a heat source in winter and a heat sink in summer.

We can simply consider the heat pump as a reverse cycle of the heat machine. The heat machine is the machine which draws heat from the high temperature environment while transfers it to the low temperature environment and employs the outside by doing this operation. The heat pump, on the other hand, is the machine that supplies the heat taken from the low temperature heat source to the high temperature environment by providing energy from the outside.[1] The main elements of a simple heat pump are two compressors, expansion valves, evaporators, and two heat exchangers called condensers.[1]

Groundwater is a suitable medium for storing solar heat. It gives the advantage of providing a constant temperature between +7 and +12°C even in cold winter days. Since the temperature level of the heat source remains constant, the heat pump's performance coefficient would be high all year round.[2] When surface waters such as lakes and rivers are used as the water source, the temperature varies more than well water, but not as much as the air. It is also an advantage that surface waters do not fall below (0°C) in our country.

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The first principle of the heat pump, which operates according to the same thermodynamic cycle as the cooling machine, was introduced by Carnot in 1824. Its implementation was carried out in 1850, when Lord Kelvin proposed the use of cooling devices for heating.[3] In this system, where Lord Kelvin uses air as a working fluid, outdoor air is drawn into a cylinder where it is expanded to reduce both temperature and pressure. The air is then expanded by sending it to a heat exchanger which is placed outside in order to allow the cooled air to draw heat from the outside. The heated air is again compressed to normal atmospheric air pressure and is delivered to the room. However, since it is compressed, its temperature is higher than normal atmospheric temperature. Lord Kelvin stated that the device, which he called “heat booster”, produces heat with 3% of the energy which is given directly to combustion. Later, as a result of the researches done by many scientists and engineers in a period of about 80 years, the heat pump became applicable in comfort heating.[3]

In the USA, the interest in Ground Source Heat Pumps technology surfaced between 1940 and 1950. At the time, research could be done in a limited way with inappropriate pipe materials. In addition, the need for the development of the heat pump was not much due to the relatively inexpensive natural gas. Due to research, technology accelerated again in Europe during the 1973 oil embargo which, in turn, led to the launch of a research program at Oklahoma State University a few years later.[3]

Further, some of the US power companies, with an aim to increase residents, use Ground Source Heat Pumps for the space heating / cooling purposes and reduce peak loads in the electrical systems which, in turn, helps conduct monetary incentive programs. The "Geothermal Heat Pump Consortium" created six-year programs of $ 100 million in order to increase Ground Source Heat Pumps sales from 40,000 to 400,000 annually which led to reducing greenhouse gas emissions by 1.5 million tons of carbon equivalent annually.[3]

There have been many studies and researches over the subject.[1-27] Water source heat pumps can be a potential technology in both residential and commercial applications, as they have outstanding heating performance. In this study, a small experimental prototype water source heat pump was tested using the Taguchi method to improve the heating performance of the water source heat pump system. In addition, R830 was used as the refrigerant in this system. The coefficient of performance (COP) was done based on Taguchi method and the best parameters were determined.

## METHOD

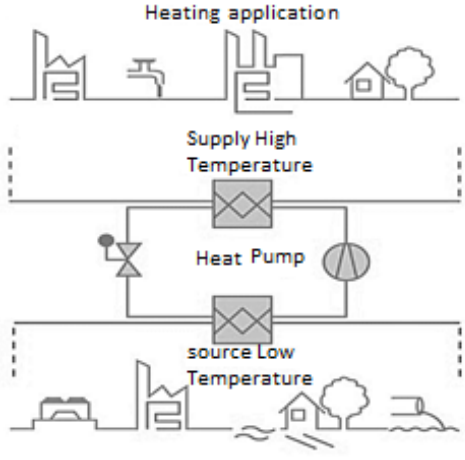
### Working principle of the heat pumps

The heat and work concepts and the forms of the energy are measured in the same units, although they are very different in nature.

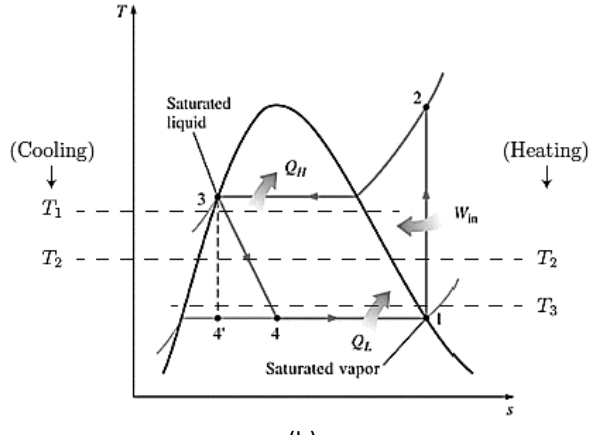
Heat is the transfer of energy between the system and the environment due to the difference in temperature and Work is not simply an influence of force. It is the transfer of energy to a system that results in a displacement against a resisting force.

Thermal energy typically flows in the direction of decreasing temperature, as per our experience. In simpler terms, we are aware that heat naturally moves from a high-temperature environment to a low-temperature one. This phenomenon occurs spontaneously without the need for any machinery. On the contrary, there is a reverse scenario where heat transfer from a low-temperature environment to a high-temperature environment does not occur spontaneously.

When thermal energy is transferred from a low-temperature environment to a high-temperature environment, then the thermal machine is referred to as a heat pump, particularly when the intended purpose is heating. To visualize functioning of a heat pump, the operation of a common refrigerator that is placed at a window of a house facing outside as its door open to the external environment, can be considered. In this case, the refrigerator would absorb heat from the outside air, which is colder than the inside of the house, convey it into the house. The operational principle of heat pumps is illustrated in Figure 1. The objective is to increase the temperature of the warm environment, by drawing the $Q_l$ heat from colder environment and imparting the $Q_h$ heat into the warm environment, consuming the work W.




(a)




(b)

**Fig. 1.** Schematic view **(a)** and T-S diagram **(b)** of vapor-driven heat pump cycle.

The most important characteristic of a heat pump is the coefficient of performance (COP), which is defined as follows:

$$\text{COP} = \frac{(\text{set point is to be obtained})}{(\text{value required to be spent})} = \frac{(\text{cooling effect})}{(\text{work input})}$$

There are many theoretical cycles, but reverse Carnot cycle is the most ideal heat pump cycle (Figure 2).

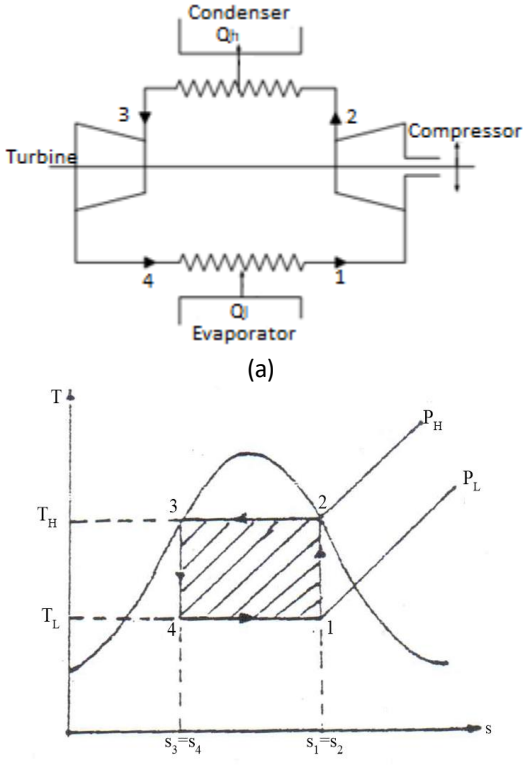



**Fig. 2.** Schematic view **(a)** and T-S diagram **(b)** of vapor-driven reversed Carnot Cycle

The ideal vapor compression cycle is used in refrigeration and air conditioning systems. It consists of four main processes: compression, condensation, expansion, and evaporation (Figure 3). In the compression stage, between 1 – 2, the refrigerant vapor is compressed adiabatically and isentropically to a high pressure and temperature and gains enthalpy. In condensation stage, between 2- 3, the high-pressure, high-temperature refrigerant vapor is condensed into a liquid by rejecting heat to the surroundings at constant pressure and loses enthalpy and temperature. In the third stage between 3 – 4, expansion occurs and the high-pressure liquid refrigerant is throttled through an expansion valve to reduce its pressure and temperature. This process causes a decrease in enthalpy and pressure. In the evaporation stage, which is the last stage of the cycle between 4 – 1, the low-pressure, low-temperature liquid refrigerant evaporates into a vapor by absorbing heat from the surroundings at constant pressure, resulting in an increase in its enthalpy and temperature.

The cycle is visualized through P-h or T-S diagrams shown in Figure 3. The P-h diagram is a plot of pressure against enthalpy whereas a T-S diagram is a plot of temperature against entropy. In these diagrams, the compression and condensation processes are represented by vertical lines and the expansion and evaporation processes are represented by horizontal lines.

Real vapor compression cycles fall short of the ideal cycle in that real cycles experience friction, pressure drops, and non-isothermal processes (Figure 4). These factors reduce efficiency compared to the ideal cycle's perfect operation. Incomplete evaporation in real systems can lead to wet compression, which further reduces efficiency and can damage the compressor. Real-world factors like ambient temperature and component wear can affect performance more significantly than in the ideal, controlled environment.

There are many problems in the process of the wet compression. The high-pressure fluid in the expansion of low-pressure wet steam with many problems in practice. At the same time, due to the enlargement of a small net loss of work entering the system, an insignificant reduction is made.

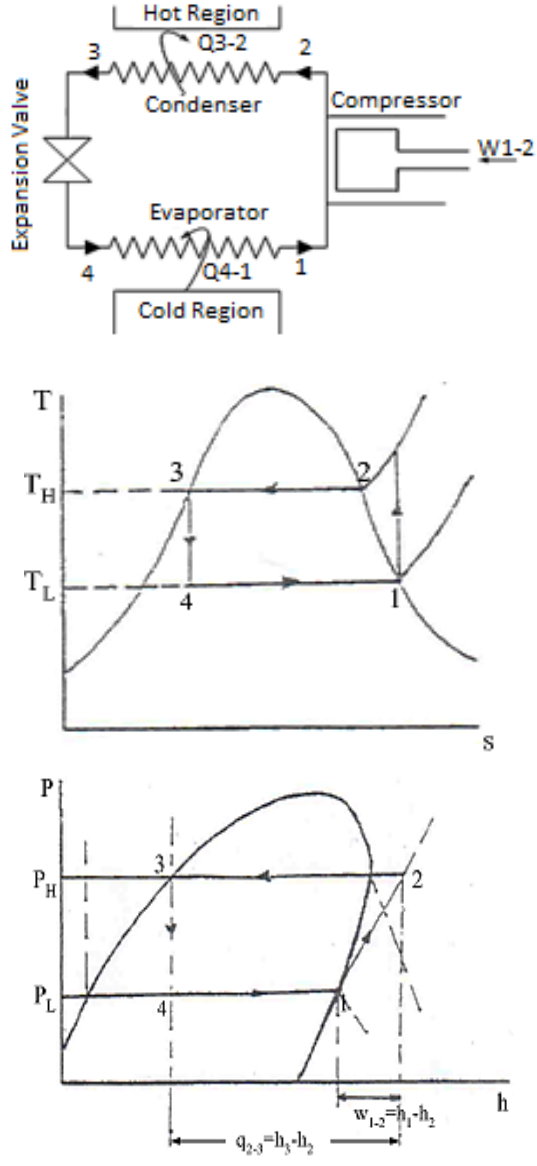



**Fig. 3.** The ideal vapor compression cycle diagram, and P-h and T-S diagrams.

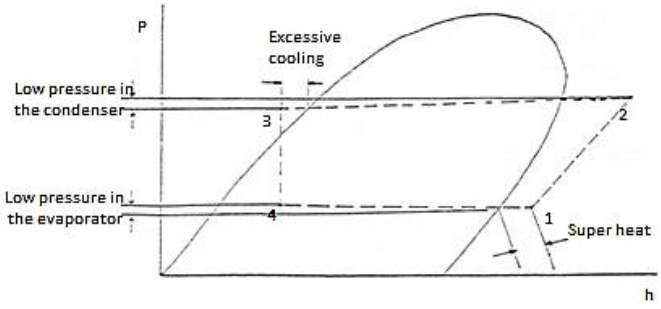



**Fig. 4.** State Change in a real cycle

Heat pumps are versatile systems that can extract heat from various sources to provide heating or cooling. Therefore, heat pumps can be used when low-temperature common heat sources are available. So, different types of heat pump applications are available based on the heat source they run on. Each type of heat pump system has its advantages and is suitable for different applications and environmental conditions. The choice of a heat source depends on factors such as climate, available space, and the specific requirements of the heating or cooling system. Some typical heat sources for heat pump cycles are as follows:

Air Source Heat Pumps (ASHP) extract heat from the ambient air, even in cold conditions. ASHPs are common in residential and commercial settings. Ground Source Heat Pumps (GSHP or Geothermal Heat Pumps) utilize the relatively constant temperature of the ground to extract or reject heat. GSHPs are highly efficient but may require more complex installation. Water Source Heat Pumps extract or reject heat using a water source, such as a river, lake, or well. Water source heat pumps are efficient but may be limited by the availability of suitable water sources. Hybrid Heat Pumps combine multiple heat sources, such as air and ground, to optimize efficiency and performance in different conditions. Absorption Heat Pumps use a heat source to drive an absorption process, typically involving a refrigerant and an absorbent. Common heat sources include natural gas, solar energy, or waste heat. Gas-fired Heat Pumps combine conventional vapor compression technology with a gas burner, often using natural gas or propane as a supplementary heat source. Solar Thermal Heat Pumps integrate solar collectors to absorb sunlight and generate thermal energy, which is then used as a heat source for the heat pump cycle. Waste Heat Recovery Heat Pumps extract heat from industrial processes, machinery, or other sources of waste heat to improve overall energy efficiency. Air-Conditioning Waste Heat Pumps capture and utilize waste heat generated during the air-conditioning process to provide additional heating.

The schematic view and photographic image of the heat pump system used in the experiments is given in Figure 5. The components and instrumentation of the system is as described below:

**Compressor:** A hermetically sealed compressor with oil bath cooling. The design ensures appropriate cooling, and installations are protected against excessive heat.

**Condenser:** Consisting of concentric tube bundles where water flow occurs in a ring.

**Expansion Valve:** Thermostatically controlled and balanced. R830 controls the amount of gas flow evaporator.

**Deflect Valve:** R830 keeps the gas separated from air or water.

**Evaporator**: (I) Used in the air as a Source of Heat Evaporators: Continuous tube and the outer wings. Copper / aluminum galvanized steel construction. For the drops of condensation, the air fan is provided with a cap. (II) Evaporators Heat as a Source of Water Used in: surrounded by the concentric tubes. Water flow is in the ring.

**Non-return valves**: Prevents the rotation of the evaporator flow which is ideal.

Consolidated Electric Power Measurement Device: A device for measuring the power which is drawn by the compressor.

**Water Flow Measurement System**: Respectively measure the water flow rate through the evaporator and condenser.

**Flowmeter R830 Gas Flow meter**: R830 measures the amount of gas mass flow.

**Thermometers**: Glass temperature measurement points in the regional instruments do this.

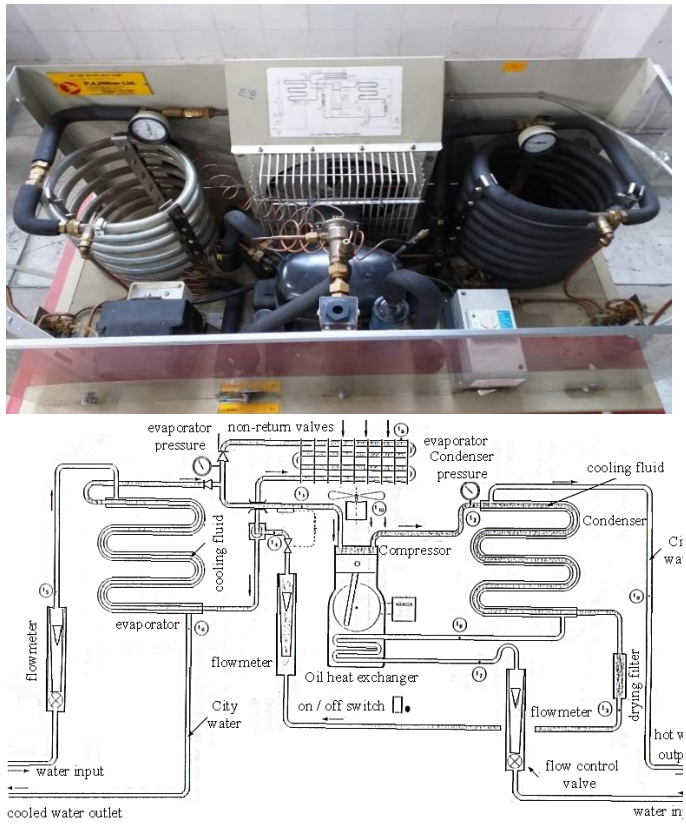



**Fig. 5.** Air and Water Source Heat Pump (photo and schematic)

**Taguchi Method**

Dr. Taguchi defined quality as the amount of damage inflicted on society by the production of defective products, and based on this, introduced the method of quality improvement called "Taguchi method". The Taguchi method is completely different from the conventional quality engineering methods. Taguchi methodology emphasizes quality design when designing products and processes. While conventional methods emphasize on inspection and quality control during the

production process or after production. In fact, Taguchi believed that the best way to improve quality was to design and create it in the product itself. The Taguchi method greatly reduces the number of experiments using orthogonal arrays (OAs), which are selected with special features from the total number of experiments in the complete factorial method.[29,30] Although the number of parameters of a Process increases, a large number of tests will be required. In the Taguchi method, it is necessary to create a special design set of factors and levels that can cover all experiments. In this study, special design sets consist of orthogonal arrays and it is necessary to use these arrays, to determine the minimum number of experiments required for the four factors at three levels shown in Table 1, and therefore to be carried out with this logic in the experiments.

To achieve the desired quality using the design, the Taguchi method simplified the following three-step process:

**Systems Design:** The focus of the system design phase is on determining the appropriate working levels of design factors, which includes designing and testing a system based on the engineer's diagnosis of the nominal process parameters based on the desired technology. System design helps determine the working levels of design factors.

**Parameter design:** Parameter design is concerned with determining the levels of factors that create the best performance of the process under consideration.

**Tolerance design:** A step used to fine-tune parameter design results. At this stage, the output quality will increase due to limiting the tolerance of factors that have a major impact on process quality.

In other words, The Taguchi method is designed to improve the quality of products and processes in which system performance depends more on factors. In planning experiments and development strategies, simple logic is usually used to create all possible combinations of factors with acceptable ranges in each of the relevant factors.

In engineering projects that have a large number of effective factors, the number of possible combinations is very high. In addition, in certain projects we may need to examine the interactions between the influencing factors. The conventional method of reducing the number of test compounds is to use partial factorial experiments. Taguchi has developed a special set of general designs for factorial experiments that cover most applications. Orthogonal arrays are part of this set of designs. Using these arrays helps us determine the minimum number of tests required for a set of factors.

Orthogonal arrays provide instructions for partial factorial experiments that include a number of test combinations. When all factors are a mixture of the surfaces in question and the interference of the factors is insignificant, standard orthogonal arrays will meet most of the test design needs. Modification of orthogonal arrays is inevitable if there is a mixture of surfaces and interactions.

Using an orthogonal array to design the experiment and the effect of multiple controllable factors on average quality characteristics and variations, while using a signal-noise (S / N) ratio to analyze experimental data, helped designers come to conclusions economically and quickly.[30]

**Table 1.** Control parameters and levels

| | Parameters | Levels | | |
|---|---|---|---|---|
| | | **1** | **2** | **3** |
| A | Evaporator water mass flow rate (g/s) | 50 | 35 | 20 |
| B | Condenser water mass flow rate (g/s) | 18 | 12 | 6 |
| C | Evaporator water inlet temperature (C) | 15 | 16 | 17 |
| D | Condenser water inlet temperature (C) | 15 | 16 | 17 |

The degree of freedom (DOF) that is defined separately for each of the input parameters and is equal to the number of levels minus one. Also, the degree of total freedom is equal to the number of trials minus one, and the degree of error freedom is equal to the difference between the total degree of freedom and the sum of the degrees of freedom of the inputs. DOF while related to a process can be calculated for each factor and each interaction as follows:

*DoF=(number of levels-1) for each factor + (number of levels-1)*(number of levels-1) for each interaction +1*

Heat transfer occurs from high-temperature regions to low-temperature regions. The first law of thermodynamics states that the total energy in a closed system remains constant. In this case, the heat transferred from the high-temperature region can be used for work or be disposed of as waste heat. The Reverse Carnot Cycle offers a theoretical ideal for refrigeration. By analyzing this cycle, we can derive a formula for COP that represents the maximum achievable efficiency for a refrigerator or heat pump operating between specific temperature reservoirs.

Accordingly, for the Reverse Carnot Cycle, $COP_h$ is:

$$COP_H = \frac{T_H(s_2 - s_3)}{(T_H - T_L)(s_2 - s_3)} = \frac{T_H}{T_H - T_L} \quad (1)$$

Vapor Compression Cycle is achieved while the reversible operations in the reverse Carnot cycle cannot be performed. Through P-h diagram,

$$W_{1-2} = h_2 - h_1 \quad \& \quad Q_{2-3} = h_2 - h_3 \quad (2)$$

$$q_{4-1} = h_1 - h_4 \quad (3)$$

$$COP_H = \frac{h_2 - h_3}{h_2 - h_1} \quad (4)$$

Real vapor compression cycle is the reverse of the adiabatic compression. The reason for that is the effect of the compressor in addition to the heat transfer and friction.

Table 2 shows an L27 orthogonal array with 27 rows which is

related to the number of parameter combination. After we analyze the experimental results, we see that the variability in the performance of the product goes through a reduction by using control parameters to the target value by using the adjustment parameter(s). It can be said that if all factor interactions in experiment systems, which include three factors at three levels, are considered, L27 orthogonal array would be the most appropriate experiment plan. Further, it is possible for us to use it effectively to regression and correlation analysis as it includes all combinations of factors and levels. We see a new experimental design method, such as orthogonal array (OA), performance statistics (signal-to-noise ratio) in Taguchi method which is different from traditional methods. In this study, we have 27 experiments (each row in the L27 orthogonal array) and assigned the columns of OA to factors and their interactions. We should determine optimum working using experimental data and provide the same or very close performance values (COP and energy efficiency) in similar or different working environments.

Taguchi method uses a special design of orthogonal arrays to study the entire parameter space with a small number of experiments only. The experimental results are then transformed into a signal to noise ratio (SNR). Taguchi recommends the use of the SNR to measure the quality characteristics deviating from the desired values. Usually, there are three categories of quality characteristic in the analysis of the SNR, i.e. the-lower-the-better, the-higher-the-better, and the nominal-the-better. The SNR for each level of process parameters is computed based on the SNR analysis. Regardless of the category of the quality characteristic, a greater SNR corresponds to superior quality characteristics. Therefore, the optimal level of the process parameters is the level with the greatest SNR. All experiments are conducted as shown in experimental plan in Tables 2&3. Contribution ratios of all factors on the performance criteria are defined depending on the SNR as given in Tables 2&3. The optimal combination of the process parameters can be predicted. Finally, an experiment of confirmation is conducted to verify the optimal process parameters which are obtained from the parameter design.

**Table 2.** Orthogonal array for L27 design

| Experimental NO | Parameters and their levels | | | | | | COP | | SNR |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E (empty) | F (empty) | Repetition 1 | Repetition 2 | |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.945447 | 1.64440 | 4.98920 |
| 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2.115925 | 2.27076 | 6.80589 |
| 3 | 1 | 1 | 1 | 1 | 3 | 3 | 2.682511 | 3.38989 | 9.46940 |
| 4 | 1 | 2 | 2 | 2 | 1 | 1 | 2.948255 | 1.00241 | 2.55609 |
| 5 | 1 | 2 | 2 | 2 | 2 | 2 | 3.118732 | 1.41998 | 5.23776 |
| 6 | 1 | 2 | 2 | 2 | 3 | 3 | 3.685319 | 2.16606 | 8.43503 |
| 7 | 1 | 3 | 3 | 3 | 1 | 1 | 3.354392 | 0.45909 | 3.83242 |
| 8 | 1 | 3 | 3 | 3 | 2 | 2 | 3.920979 | 0.66787 | 0.62007 |
| 9 | 1 | 3 | 3 | 3 | 3 | 3 | 3.920979 | 1.04091 | 3.06283 |
| 10 | 2 | 1 | 2 | 3 | 1 | 2 | 2.348075 | 1.64440 | 5.59730 |
| 11 | 2 | 1 | 2 | 3 | 2 | 3 | 2.744685 | 2.27076 | 7.86908 |
| 12 | 2 | 1 | 2 | 3 | 3 | 1 | 2.22874 | 3.38989 | 8.41126 |
| 13 | 2 | 2 | 3 | 1 | 1 | 2 | 1.481147 | 0.91817 | 0.85652 |
| 14 | 2 | 2 | 3 | 1 | 2 | 3 | 1.877758 | 1.33574 | 3.74644 |
| 15 | 2 | 2 | 3 | 1 | 3 | 1 | 1.361813 | 2.08183 | 4.14566 |
| 16 | 2 | 3 | 1 | 2 | 1 | 2 | 2.183113 | 0.54813 | 2.47745 |
| 17 | 2 | 3 | 1 | 2 | 2 | 3 | 2.579723 | 0.75692 | 0.23263 |
| 18 | 2 | 3 | 1 | 2 | 3 | 1 | 2.063779 | 1.12996 | 2.93289 |
| 19 | 3 | 1 | 3 | 2 | 1 | 3 | 1.474128 | 1.50361 | 3.45585 |
| 20 | 3 | 1 | 3 | 2 | 2 | 1 | 1.179302 | 2.12996 | 3.28153 |
| 21 | 3 | 1 | 3 | 2 | 3 | 2 | 1.247493 | 3.24910 | 4.33385 |
| 22 | 3 | 2 | 1 | 3 | 1 | 3 | 1.56438 | 1.09627 | 2.07364 |
| 23 | 3 | 2 | 1 | 3 | 2 | 1 | 1.273566 | 1.51384 | 2.78647 |
| 24 | 3 | 2 | 1 | 3 | 3 | 2 | 1.341757 | 2.25993 | 4.25241 |
| 25 | 3 | 3 | 2 | 1 | 1 | 3 | 1.073004 | 0.54813 | 3.21896 |
| 26 | 3 | 3 | 2 | 1 | 2 | 1 | 0.778179 | 0.75692 | 2.30038 |
| 27 | 3 | 3 | 2 | 1 | 3 | 2 | 0.84637 | 1.12996 | 0.37263 |

**Statistical analysis**

In statistical analysis, the ANOVA (Analysis of Variance) and regression are commonly used techniques to assess the relationship between variables and to determine the significance of these relationships. These methods are essential for understanding the impact of different factors on a response variable and for making informed decisions based on the data. ANOVA is used to compare the means of three or more groups to determine if there is a statistically significant difference between them. It provides insights into the variation within and between groups, allowing us to assess the impact of different factors on the response variable.

Regression analysis, on the other hand, is used to model the relationship between a dependent variable and one or more independent variables. It helps us understand how the independent variables affect the dependent variable and allows us to make predictions based on the model. Both ANOVA and regression analysis are conducted at a specific confidence level, such as 95%, to ensure that the results are statistically significant and reliable. In this study, Statistical analyses (ANOVA, regression) were performed for a 95% confidence level.

**(S/N) analysis**

Taguchi method employs a signal-to-noise ratio (S/N) to measure the present variation. The definition of (S/N) ratio differs according to an objective function, i.e., a characteristic value. There are three kinds of characteristic value: Nominal is Best (NB), Smaller is Better (SB) and Larger is Better (LB).

(S/N) ratio of LB is formulated as follows:

$$\frac{S}{N} = -10 * \log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^{\,2}}\right) \quad (5)$$

In equation 5, n is the number of measurements, the measured characteristic value, and the unit of the (S / N) ratio is decibels.

**Analysis of variance**

In the experiments of this study, the Taguchi method minimizes the variability around the target while bringing the performance value to the target value and another advantage is that the working conditions can be reproduced in the optimum real applications determined by the laboratory study. Since the partial experiment is only an example of the complete experiment, the analysis of the partial experiment includes a confidence analysis that can be placed in the results. however, a standard statistical technique called ANOVA was used.

ANOVA determines the significance of individual factors and interaction effects, so (S / N) performance statistics, (n), number of repetitions for an experimental combination, and ($Y_i$) are performance values of the experiment. The variance ratio, commonly referred to as the F statistic, is the variance ratio caused by the variance and error term caused by the effect of a factor, and this ratio was used to measure the importance of the investigated factor according to the variance of all the factors included in the error term.

To create an ANOVA table, following steps were followed:

- the total mean of the observations was calculated
- the sum of squares between (SSB), which is the variance due to the interaction between the different levels, was calculated
- the sum of squares within (SSW), which is the variance within each level, were calculated
- the sum of squares total (SST), which is the total variance in the data, was calculated
- the degrees of freedom between (DFB) and within (DFW were calculated.
- the mean square between (MSB) and mean square within (MSW) were calculated by dividing the sum of squares by the respective degrees of freedom.
- the F-statistic was calculated by dividing MSB by MSW.
- finally, the p-value was determined using the F-statistic.

As seen in Table 3, the sum of squares between groups (SSB) is very small, indicating that the variance between the different factors is minimal. The sum of squares within groups (SSW) is much larger, suggesting that there is more variance within the levels of each factor. The F-value is extremely small, and the P-value is effectively 1.0, which suggests that there is no statistically significant difference between the means of the different factors at the conventional significance levels. This implies that, based on the ANOVA, the factors do not have a statistically significant effect on the response variable. However, the practical significance should also be considered in the context of the specific domain or application.

In the Taguchi method, the experiment corresponding to the optimum working conditions might not have been undertaken during the whole period of the experimentation. In such cases, the performance value corresponding to optimum working conditions can be predicted by utilizing the balanced characteristic of the OA.

**Table 3.** ANOVA Table

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square | F-Value | P-Value |
|---|---|---|---|---|---|
| Between Groups | 1.0944444e-07 | 5 | 2.188889e-08 | 7.0704573e-09 | 0.999999 |
| Within Groups | 37.14988317 | 12 | 3.095823597 | | |
| Total | 37.14988328 | 17 | | | |

Analysis of variance (ANOVA) was used to examine the importance of trust and effect of adjustable and independent parameters on performance, and in Taguchi method, additive model was created and used to estimate the effect of control factors on response.

In Figure 7, each factor's distribution is visualized as overlaid with varying colors. The histogram provides a visual comparison of the frequency and spread of the response variable across different factors.

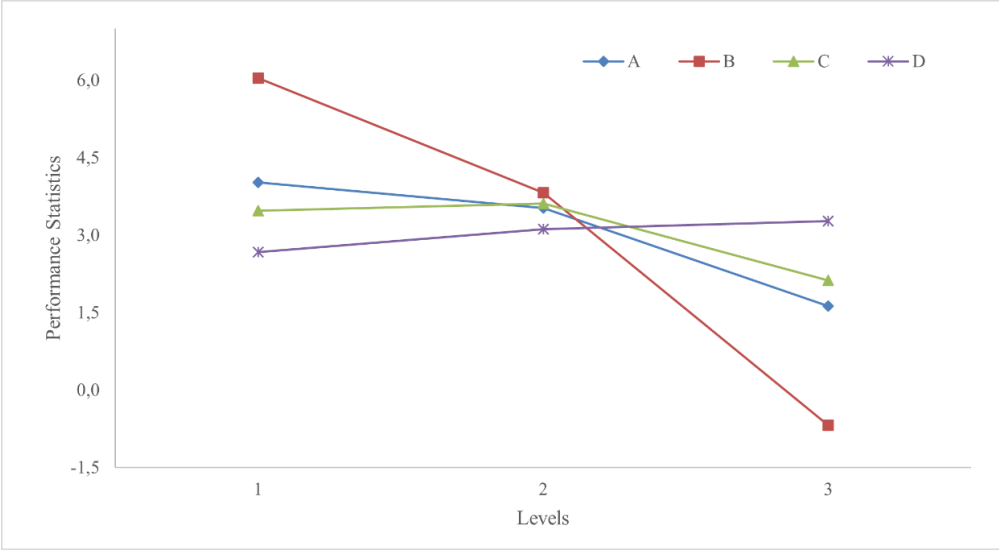



**Fig. 6.** The effect of the parameters used on the coefficient of performance (COP).

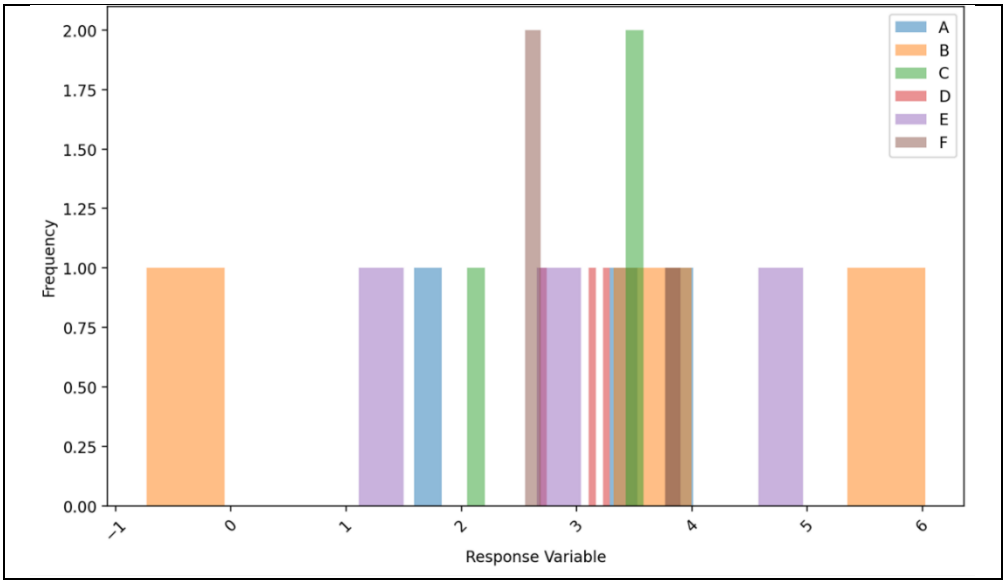



**Fig. 7.** A histogram of distribution of Response Variables by Factor

**Table 4. Variance analysis for efficiency**

| Level | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | 4.0115 | 6.0237 | 3.4517 | 2.6801 | 1.1111 | 2.5523 |
| 2 | 3.4794 | 3.7878 | 3.5794 | 3.1098 | 3.0044 | 2.6237 |
| 3 | 1.588 | -0.732 | 2.0478 | 3.2889 | 4.9634 | 3.9029 |
| Delta | 2.4235 | 6.7563 | 1.5316 | 0.6088 | 3.8523 | 1.3506 |
| Rank | 3 | 1 | 4 | 6 | 2 | 5 |

The deltas and ranks help in prioritizing which factors to focus on for further analysis or optimization. Factors with higher ranks (lower delta values) might be considered less critical to the outcome of the process or system being studied. As seen in Table 4, Factor B has the highest delta (6.7563), indicating that it has the most significant effect on the response variable. It is ranked 1, confirming its strong influence. Factor A and Factor E are the next most significant factors, with deltas of 2.4235 and 3.8523, respectively, and ranks of 3 and 2. These factors also have a considerable impact on the response variable. Factor C and Factor F have moderate deltas (1.5316 and 1.3506) and are ranked 4 and 5, indicating a lesser but still notable effect on the response variable. Factor D has the smallest delta (0.6088) and is ranked 6, suggesting it has the least impact on the response variable among the factors tested.

After the (S / N) analyzes were calculated, the (ANOVA) analysis verification tests were carried out using the experimental results, so that first the optimum parameters were selected and then, using these parameters, to predict and verify the improvements in performance characteristics. The results of validation experiments using optimum design parameters are presented in Table 5 and comparisons between estimated and actual (COP) are shown. As can be seen in Table 5, it is seen that there is a good agreement between the estimated and actual (COP) values. By comparison of the initial parameters and the optimum parameters which are obtained by the Taguchi approach, it is observed that the improvement of (S/N) ratio for (COP) value is 3.375. Using the results from validation experiments, the Taguchi approach validated in optimization of design parameters.

The boxplots illustrate the distribution of COP values obtained in repetitions across different levels of parameter B. The variation within each level of B and the median values can indicate how parameter B might influence the COP values. It provides a clear visualization of how the COP values vary across different levels of parameter B. For the COP in the first repetition, the boxplots show the median, interquartile range, and outliers for COP1 at each level of parameter B. It can be observed how the median COP value in repetition 1 changed with different levels of B, and whether there were any significant differences in the distribution across these levels. For COP in the first repetition, similarly, we can identify differences in the distribution of these two COP values with respect to parameter B by comparing the boxplots.

**Regression Analysis**

The regression analysis done has revealed that the model has a high R-squared value of 0.975, indicating that the independent variables explain a large portion of the variance in the dependent variable, COP. The F-statistic is significant with a probability of 3.47e-08, suggesting that the model is a good fit for the data. The coefficients for the independent variables A, B, C, D, E, and F have been estimated along with their standard errors, t-values, and p-values.

**Table 5.** Optimum working parameters

| Optimums | | Parameters | | | | COP | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | Prediction | Confidence interval | Real |
| COP | Level | 1c | 1b | 2d | 3a | 2.7 | 1.48 - 3.92 | 3.375 |
| | Value | 50 | 18 | 16 | 18 | | | |
| General | Level | 1 | 1 | 2 | 3 | 2.7 | 1.48 – 3.92 | 3.375 |
| | Value | 50 | 18 | 16 | 18 | | | |

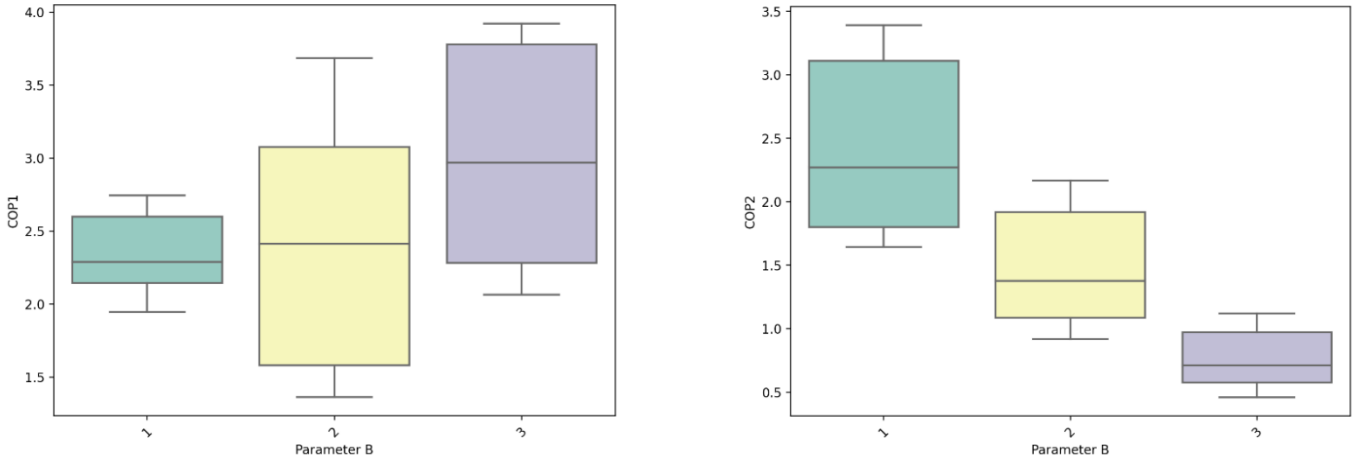



**Fig 8.** Correlation between the levels of factor B and COP values obtained in repetitions of experiments

The output of the regression analysis is presented in three tables, Table 6, Table 7 and Table 8. Table 6 provides an overview of the regression results, including the R-squared value, the F-statistic, and other statistical measures. The second table displays the coefficients, standard errors, t-values, and p-values for each independent variable, along with their 95% confidence intervals.

The R-squared value of 0.975 indicates that the model explains 97.5% of the variance in the dependent variable, COP. This suggests that the independent variables A, B, C, D, E, and F collectively have a strong explanatory power for COP.

Looking at the coefficients presented in Table 7, it can be seen that the constant term has a value of 1.8704, and the coefficients for A, B, C, and D are -0.9804, 0.1924, 0.0134, and 0.5363, respectively. The t-values and p-values provide information about the statistical significance of each coefficient. In this case, A, B, and D are statistically significant, while C is not.

Several diagnostic tests were conducted to assess the validity of the regression model and results are presented in Table 8. Omnibus/Prob(Omnibus) tests the hypothesis that the residuals are normally distributed. A lower value indicates a closer approximation to the normal distribution. In this case, the Omnibus value is 1.689 and the Prob(Omnibus) is 0.43, suggesting that the residuals could be normally distributed since the p-value is not low enough to reject the normality assumption.

Durbin-Watson diagnostics tests for the presence of autocorrelation in the residuals from a regression analysis. The value ranges from 0 to 4, with values around 2 suggesting no autocorrelation. The Durbin-Watson statistic here is 2.258, which is close to 2, indicating a low likelihood of autocorrelation.

Jarque-Bera (JB)/Prob(JB) is another test of the normality of the residuals. A large JB value indicates that the residuals are not normally distributed. Here, the JB value is 0.34 and the Prob(JB) is 0.843, which again suggests that the residuals are normally distributed.

Skewness statistic measures the asymmetry of the probability distribution of the residuals. A skewness value of 0 indicates that the residuals are perfectly symmetrical. Here, the skewness is 0.053, which is very close to 0, suggesting the residuals are fairly symmetrical. Kurtosis, on the other hand, measures the 'tailedness' of the distribution of the residuals. A kurtosis value of 3 indicates a normal distribution. The kurtosis value here is 3.665, which is slightly higher than 3, suggesting a leptokurtic distribution (with slightly heavier tails than a normal distribution).

Condition number measures the sensitivity of the model's output to its input. A high condition number indicates potential multicollinearity or other numerical problems. The condition number here is 28.4, which does not indicate a severe multicollinearity problem.

**Table 6.** Ordinary Least Squares (OLS) Regression Results

| | | | |
|---|---|---|---|
| ***Dep. Variable:*** | COP | ***R-squared:*** | 0.975 |
| ***Model:*** | OLS | ***Adj. R-squared:*** | 0.962 |
| ***Method:*** | Least Squares | ***F-statistic:*** | 72.02 |
| ***No. Observations:*** | 18 | ***Prob (F-statistic):*** | 3.47e-08 |
| ***Df Residuals:*** | 11 | ***Log-Likelihood:*** | 12.761 |
| ***Df Model:*** | 6 | ***AIC:*** | -11.52 |
| ***Covariance Type:*** | nonrobust | ***BIC:*** | -5.289 |

**Table 7.** Coefficients, standard errors, t-values, p-values, and 95% confidence intervals for each independent variable.

| | **Coefficients** | **Std Err** | **t** | **P>\|t\|** | **[0.025** | **0.975]** |
|---|---|---|---|---|---|---|
| **Const** | 18.704 | 0.202 | 9.266 | 0.000 | 1.426 | 2.315 |
| **A** | -0.9804 | 0.072 | -13.652 | 0.000 | -1.138 | -0.822 |
| **B** | 0.1924 | 0.046 | 4.150 | 0.002 | 0.090 | 0.294 |
| **C** | 0.0134 | 0.046 | 0.288 | 0.778 | -0.089 | 0.115 |
| **D** | 0.5363 | 0.046 | 11.570 | 0.000 | 0.434 | 0.638 |
| **E** | 0.0698 | 0.045 | 1.537 | 0.152 | -0.030 | 0.170 |
| **F** | 0.2816 | 0.045 | 6.200 | 0.000 | 0.182 | 0.382 |

**Table 8.** Values for several diagnostic tests

| | | | |
|---|---|---|---|
| **Omnibus:** | 1.689 | **Durbin-Watson:** | 2.258 |
| **Prob(Omnibus):** | 0.430 | **Jarque-Bera (JB):** | 0.340 |
| **Skew:** | 0.053 | **Prob(JB):** | 0.843 |
| **Kurtosis:** | 3.665 | **Condition Number** | 28.Nis |

These diagnostics indicate that the model does not violate the assumptions of linear regression significantly. Overall, the regression analysis indicates that the model is a good fit for the data, and the coefficients for A, B, and D have a statistically significant association with the dependent variable COP.

**CONCLUSION**

In this experimental study, the effect of water on the inlet and outlet flow rate and temperature was optimized with the Taguchi experimental design method and then obtained according to the analysis of the optimum parameter combination (S / N) ratio for the minimum inlet and outlet water flow, temperature and maximum heat transfer. (ANOVA) analysis using the obtained ratios and the contributions of the parameters on the performance characteristics were determined. At the end of this study, the validity of the approach of the Taguchi test method was checked by using the data of the validation experiments. The results obtained are expressed below:

• In this study, if the Taguchi method is not used 148 routine experiments may be required. however, to provide a significant advantage in both time and cost, the number of experiments was reduced to 27 after Taguchi used an experimental design.

• Optimum parameter levels for maximum heat transfer are determined as A1, B1, C2 and D3, but changes may occur in heat transfer according to Reynolds number and pressure values.

• The results of the experiments performed at the predicted values and optimum parameter levels using the Taguchi approach were compared with the results of the validation experiments.

• Quite satisfactory and close agreements appear between the predicted and experimental values, and this demonstrated that the Taguchi approach has been successfully used to determine optimum design parameters for heat pumps.

• The Taguchi method is a strategy to improve the quality of the process and achieve a reinforced product using the experimental design method and can be used in most heat transfer tests.

The study successfully applied the Taguchi method to determine the optimal conditions for high yields of heat water while it was validated by running a four-factor, three-level experimental design. The collected data were analyzed by using ANOVA-TM computer software package for evaluation of the effect of each parameter on the optimization criteria. The results obtained for investigating the performance of the heat pump after conducting the experiments are summarized as follow:

•The contributions of all the working parameters (heat input, fluid mass and flow rate) in heat pump performance have equal importance.

•The overall heat transfer coefficient of heat pipe of pump is almost same for all levels.

•Taguchi optimal solutions give the better results for heat pump operations while it also reduces the number of experiments that are required for finding its performance metrics.

•The experimental results show that the heat of water input and flow rate plays an important role in the operations of heat pipe and these contributions are almost equal.

The numerical value of the maximum point in each graph shows the best value of that particular parameter which is given in Table 5 for each one. Further, they indicate the optimum condition in the range of the experimental conditions. It may be stated that as the optimum conditions determined by the Taguchi method in a laboratory environment are also reproducible in the real production environments, the findings of the present laboratory-scale study may be very useful for heat pump applications in the industrial scale. The raw water source provides a favorable heat source compared to an ambient air source except for the time of spring as the ambient air temperature is higher than the raw water temperature by 18 ◦C during this time. In spring and autumn, the heating and cooling loads are extremely low which is the main reason of the poorer performance of the raw-water heat pump system in these seasons. The average unit COP in the heating season is low while the average unit COP for the cooling season increases.

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**REFERENCES**

1. Acuña J, Palm B. A novel coaxial borehole heat exchanger: description and first distributed thermal response test measurements. In: *Proceedings of the World Geothermal Congress*; 2010; Bali, Indonesia. 2010.
2. Develioğlu, M. *Yer Kaynaklı Isı Pompalarının Teknolojik Gelişimi ve Türkiye'deki Uygulanabilirliği*. [MS thesis]. Fen Bilimleri Enstitüsü, Hacettepe University, 2012.
3. Unlu K. *Hava ve toprak kaynaklı ısı pompalarına etki eden parametrelerin incelenmesi*. [PhD thesis]. Bursa Uludağ University; 2005.
4. Ajah A, Mesbah A, Grievink J, Herder P, Falcao P, Wennekes S. On the robustness, effectiveness and reliability of chemical and mechanical heat pumps for low-temperature heat source district heating: A comparative simulation-based analysis and evaluation. *Energy*. 2008;33(6):908-929. doi:10.1016/j.energy.2007.12.003
5. Bendell A, Disney J, Pridmore WA. *Taguchi Methods*. New York, NY: Springer-Verlag; 1989.
6. Baek NC, Shin UC, Yoon JH. A study on the design and analysis of a heat pump heating system using wastewater as a heat source. *Sol Energy*. 2005;78(3):427-440. doi:10.1016/j.solener.2004.07.009

7. Purkayastha B, Bansal PK. An experimental study on HC290 and a commercial liquefied petroleum gas (LPG) mix as suitable replacements for HCFC22. *Int J Refrig*. 1998;21(1):3-17. doi:10.1016/s0140-7007(97)00083-2
8. Can M. Endüstriyel atık akışkanların değerlendirilmesi ve ülke ekonomisine katkısı. *Ekoloji Dergisi*. 1995;17: Ekim, Kasım, Aralık.
9. McMullan JT. Refrigeration and the environment — issues and strategies for the future. *Int J Refrig*. 2002;25(1):89-99. doi:10.1016/s0140-7007(01)00007-x
10. Huang K, Wang H, Zhou X. Heat pump for high school bathroom heat recovery. *Renew. Energy Resour. Greenery Future*. 2006;8(12-1):1-6.
11. Correa G, Almanza R. Copper based thin films to improve glazing for energy-savings in buildings. *Sol Energy*. 2004;76(1-3):111-115. doi:10.1016/j.solener.2003.08.014
12. Kotcioglu I, Cansiz A, Khalaji MN. Experimental investigation for optimization of design parameters in a rectangular duct with plate-fins heat exchanger by Taguchi method. *Appl Therm Eng*. 2013;50(1):604-613. doi:10.1016/j.applthermaleng.2012.05.036
13. Kotcioglu I, Khalaji MN, Cansiz A. Heat transfer analysis of a rectangular channel having tubular router in different winglet configurations with Taguchi method. *Appl Therm Eng*. 2018;132:637-650. doi:10.1016/j.applthermaleng.2017.12.120
14. Yu X, Wang RZ, Zhai XQ. Year round experimental study on a constant temperature and humidity air-conditioning system driven by ground source heat pump. *Energy*. 2011;36(2):1309-1318. doi:10.1016/j.energy.2010.11.013
15. Lazzarin RM. Heat pumps in industry II: Applications. *Heat Recov Syst CHP.* 1995;15(3):305-317. doi:10.1016/0890-4332(95)90014-4
16. Nuntaphan A, Chansena C, Kiatsiriroat T. Performance analysis of solar water heater combined with heat pump using refrigerant mixture. *Appl Energy.* 2009;86(5):748-756. doi:10.1016/j.apenergy.2008.05.014
17. Sen M, Yang KT. Applications of artificial neural networks and genetic algorithms in thermal engineering. *CRC Handbook of Thermal Engineering*. 2000:620-661.
18. Chen C, Sun FL, Feng L, Liu M. Underground water-source loop heat-pump air-conditioning system applied in a residential building in Beijing. *Appl Energy*. 2005;82(4):331-344. doi:10.1016/j.apenergy.2004.12.002
19. Park KJ, Shim YB, Jung D. Performance of R433A for replacing HCFC22 used in residential air-conditioners and heat pumps. *Appl Energy*. 2008;85(9):896-900. doi:10.1016/j.apenergy.2007.11.003
20. Medrano M, Bourouis M, Coronas A. Double-lift absorption refrigeration cycles driven by low–temperature heat sources using organic fluid mixtures as working pairs. *Appl Energy*. 2001;68(2):173-185. doi:10.1016/s0306-2619(00)00048-9
21. Koo KM, Jeong YM, Hwang YJ, Lee JK, Jang SY, Kim IK, Lee DH. Heating performance of a ground source heat pump system through actual operation. *Proceedings of the SAREK Conference*. The Society of Air-Conditioning and Refrigerating Engineers of Korea; 2008.
22. Yamazaki T, Kubo Y. Development of a high-temperature heat pump. *Newsl IEA Heat Pump Cent*. 1985;3(4):18-21.
23. Pinar AM, Uluer O, Kırmaci V. Optimization of counter flow Ranque–Hilsch vortex tube performance using Taguchi method. *Int J Refrig*. 2009;32(6):1487-1494. doi:10.1016/j.ijrefrig.2009.02.018
24. Yun JY, Lee KS. Influence of design parameters on the heat transfer and flow friction characteristics of the heat exchanger with slit fins. *Int J Heat Mass Transfer.* 2000;43(14):2529-2539. doi:10.1016/s0017-9310(99)00342-7
25. Nakayama W. A methodology to work on geometrically complex heat transfer systems: the cases of heat conduction through composite slabs. *Int J Heat Mass Transfer*. 2003;46(18):3397-3409. doi:10.1016/s0017-9310(03)00136-4
26. Williamson SS, Emadi A. Comparative Assessment of Hybrid Electric and Fuel Cell Vehicles Based on Comprehensive Well-to-Wheels Efficiency Analysis. *IEEE Trans Veh Technol*. 2005;54(3):856-862. doi:10.1109/tvt.2005.847444
27. Li YL, Zhang XS, Yin YG. Studies on solid desiccant integrated natural gas engine-driven air conditioning system. *Proceedings of the 10th International Conference on Indoor Air Quality and Climate*, vols. 1–5. 2005:1279–1283.
28. Nam Y, Ooka R. Numerical simulation of ground heat and water transfer for groundwater heat pump system based on real-scale experiment. *Energy Build*. 2010;42(1):69-75. doi:10.1016/j.enbuild.2009.07.012
29. Nam Y, Ooka R, Shiba Y. Development of dual-source hybrid heat pump system using groundwater and air. *Energy Build*. 2010;42(6):909-916. doi:10.1016/j.enbuild.2009.12.013
30. Esme U. Application of Taguchi method for the optimization of resistance spot welding process. *Arab J Sci Eng.* 2009;34.

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# Comparison of C# and Python programming languages in terms of performance and coding on SQL server DML operations

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## ABSTRACT

Nowadays, there are several computer programming languages and database management systems, and they have advantages and disadvantages one over another. Databases are essential components of computer programs, regardless of their language. Thanks to databases, computer programs record the data that they need or produce and perform the given tasks by retrieving these data when necessary. For a computer program to run efficiently and quickly, it is essential that both the database and the computer program are well structured. In this study, C# and Python languages, which are among the most widely used programming languages out of these various programming languages, have been evaluated in terms of transaction performance and the amount of code that needs to be written to perform SQL DML (Data Manipulation Language) operations such as INSERT, SELECT, UPDATE, DELETE operations and print the results of the operation on the screen via MSSQL database,[1] which is one of the most widely used database management systems. In terms of processing performance, it was observed that neither programming language provided a significant superiority over the other, although mathematically Python language seems to have performed better when looking at the processing times. In terms of code size and readability, although C# is generally considered to be a more readable language in terms of code readability, in the context of the programs written for this study, there was no difference between the two programming languages in terms of code readability. In terms of code size, Python provided a clear superiority. As a result, it has been determined that both languages have superior features compared to each other, and that there is no definite superiority between these two languages that can be a reason for preference over each other in DML operations. The choice of language should be based on the requirements of the project, the ecosystem and the skills of the team.

**Keywords:** *C#, Python, MSSQL, DML Operations, Code Writing, Performance*

## INTRODUCTION

Today, information and communication technologies such as computers, mobile devices, the internet, and many other digital communication tools are widely used, and these technologies deeply affect social, cultural, economic, and social life. One of the first concepts that comes to mind when it comes to information and communication technologies is data, and the other is computer software. For this reason, storing data in a database system through computer software, and retrieving, correcting, or deleting data when necessary constitutes the basis of information and communication technologies.

In modern software development processes, performing database operations effectively and efficiently is a critical factor affecting the performance and reliability of applications. Therefore, the choice of programming language has a direct impact on the effectiveness of the tools used to perform database operations. C# and Python are currently among the most widely used programming languages, and both are used to interact with relational database management systems such as SQL Server. This study aims to make a comparison between C# and Python programming languages in terms of performance and code writing efficiency in SQL Server Data Manipulation Language (DML) operations.

One of the main goals of this study is to compare the performance of C# and Python in SQL Server DML transactions, to determine the impact of each language in terms of transaction execution time, and to evaluate the impact of each language in terms of performance and code quality, such as code readability, flexibility, and error handling capabilities.

Based on the results obtained, it will be possible to identify the advantages and disadvantages of C# and Python in the creation and execution of queries for SQL Server DML transactions and analyze whether each language is more suitable in specific use cases.

## DATABASE MANAGEMENT SYSTEMS

Today, human life is intertwined with information technologies and there is almost no area where information devices are not available. From daily activities to working life, from health to entertainment, computers, software and therefore databases and computer programs are integrated with human beings. This integration has led to many differences in the quantity and quality of data to be stored and used, and in the way data is retrieved. This has led to different approaches and systems

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in database systems and data models.

Database Management System (DBMS) is a collection of data categorized in a table or several tables integrated with each other and an application program that regulates how to access this data.[2] This collection of data is often called a database. The main purpose of DMS is to provide a way to quickly store and retrieve the information in the database. Databases allow stored data to be queried in relation to other data that is related to that data. In a relational database, data is stored in the form of relationships or two-dimensional tables, and there is a data relationship between the tables.[3]

While the amount of data to be stored is increasing day by day, the structure of the data to be stored is of great importance in database design. In addition to the structured data stored in a certain template, the widespread use of the internet today has disrupted the traditional data structure and led to the emergence of unstructured databases. In this context, it would be more accurate to examine the database model in two groups: relational databases, where structural data is stored within a template, and non-relational databases, where data that is not within a certain template is stored, or, in other words, NoSQL databases, which do not have to be relational.

**Relational Database Systems**

Relational Database Systems (RDMS) Organization based on the relational data model, first proposed by Edgar Frank Codd in 1970 are various software systems used to manage database systems.[4] Although traditional database systems such as the network data model and the hierarchical data model were used before relational databases, their use has declined with the development and more widespread use of relational database systems.

Relational database systems are the most preferred database management system for storing structured data whose structure is defined on a specific template.[4] In relational database systems, data is stored in tables consisting of rows and columns. Each transaction in relational databases is defined as a transaction, and these transaction operations work in an all-or-nothing context. Each transaction must have basic properties known as ACID. Transactions that do not have ACID basic properties are not executed and are rolled back. ACID stands for Atomic, Consistent, Isolated, and Durable.[5]

ACID principles;[5]

- Atomic: guarantees the successful completion of an operation in the database, such as Insert, Update or Delete. The initial operation cannot be split; it must either be committed, i.e., applied, or rollback, i.e., canceled.
- Consistent: it guarantees that the data stored in the database conforms to the predetermined template, that is, its consistency. When a transaction is committed or rollbacked, the database must maintain its consistency. If the transaction completes successfully, the changes are applied to the database, if it fails, it is automatically rolled back.
- Isolation: each transaction operation is performed in isolation, meaning that no other transaction operation can access the result of this transaction operation until the transaction is finalized.
- Durable: when a transaction is successfully completed, i.e., committed, the result of the transaction is persistent in the database, no matter what.

The most popular relational databases worldwide are Oracle, MySQL, Microsoft SQL Server and PostgreSQL.[6]

**NoSQL Database Systems**

NoSQL database Systems process large volumes of rapidly changing data in unstructured form in ways that differ from a relational database containing rows and tables. NoSQL technologies have been used under various names since the 1960s. Due to the changes in the data environment, the popularity of NoSQL database systems is increasing day by day as software developers have to work with large volumes and a wide variety of data generated by cloud technologies, social media platforms, and mobile devices and have to adapt to change.[7]

Data from different types of data-sending sources, such as social media applications, third-party databases, mobile devices, and smart sensors, is not suitable for storage in a relational model database system, and this is where non-relational NoSQL database systems come into play.[8]

The term "Not only SQL" is a phrase often used for NoSQL databases. This term emphasizes an approach that is not limited to SQL queries only, as in traditional relational database systems. In this way, it aims to take full advantage of the flexibility that NoSQL databases offer.

NoSQL database systems operate on the basis of certain principles to meet the requirements of big data and distributed systems, called BASE which represent the following concepts.[9]

- Basically Available: This principle emphasizes the flexibility of the system. NoSQL databases usually offer extensibility at a high scale and provide extensibility with the flexibility to work in the system. Certain data records may sometimes be temporarily unavailable, but generally, the system will be functional.
- Soft state: This principle refers to consistency and flexibility. NoSQL databases offer a more flexible approach to consistency and do not require immediate synchronization between copies of data. This is critical for expansion on a larger scale.
- Eventually Consistent: This policy complements the consistency and flexibility of NoSQL databases. It states that consistency will eventually be achieved after changes, but this process may not happen instantly. In this case, there may be temporary inconsistency between copies of data, but eventually the system will reach the desired state of consistency.

These features demonstrate that NoSQL databases offer a different approach than traditional relational databases and are better suited to modern applications that require scalability and flexibility.

**NoSQL Database Types**

- Document-based NoSQL Databases: These types of databases are used to store and query data in the form of documents. For example, MongoDB stores documents in JSON or BSON format. These types of databases usually allow flexible data structures and dynamic schemas.
- Column-based NoSQL Databases: Column-based databases are ideal for large amounts of horizontally scaled data. Examples such as HBase and Cassandra are column-based databases. They are designed to provide high performance and scalability.
- Key-value based NoSQL Databases: These types of databases store data in the form of simple key-value pairs and are ideal for applications that require fast access. Databases such as Redis and Amazon DynamoDB are key-value-based NoSQL solutions.
- Graph-based NoSQL Databases: These databases are used to store and query relational data. Neo4j is an example of this type of database. Graph-based databases are ideal for effectively managing complex relationships and connections.
- Each type of NoSQL offers different advantages for specific use cases and requirements. It is important to choose the most suitable one according to the needs of the application and the data structure.
- The most popular relational databases worldwide are MongoDB, Redis, Cassandra, Neo4j .[1,10]

**What Are The Most Used DMSs?**

Stack Overflow, a question-and-answer website about computer programming with more than 50 million users worldwide, published the following graph about the most commonly used DMSs in the most popular technologies section of the "Developer Survey" conducted in 2022.

As seen in this survey of 63,327 computer programmers (Fig 1), in which participants can choose more than one database system, the database systems with the highest preference rates are MySQL, PostgreSQL, SQLite, MongoDB and Microsoft SQL Server.

**Python and C# Programming Languages**

**Python Programming Language**

**Python Overview**

Python is a general-purpose, interactive, and high-level programming language. It was started to be developed by Guido van Rossum in 1991. Its basic philosophy is code readability and simplicity.[11]

Python is a programming language that has been accepted worldwide and has had a wide user base since its first release. It is used both in academia and industry, from scientific research to financial analysis.

Technology giants frequently use Python in areas such as product development and data analysis. Python's popularity is also supported by the fact that it has become part of university courses. Its use as a core language in programming courses encourages students to learn Python and familiarize themselves with its use in their future professional lives.

Python's powerful standard library and wide module support make it usable in a variety of fields. Thanks to these features, it can be used effectively in data analysis, web development, machine learning, and artificial intelligence, and many more. Python's flexibility and easy readability make it preferable for both experienced developers and beginners.[12]

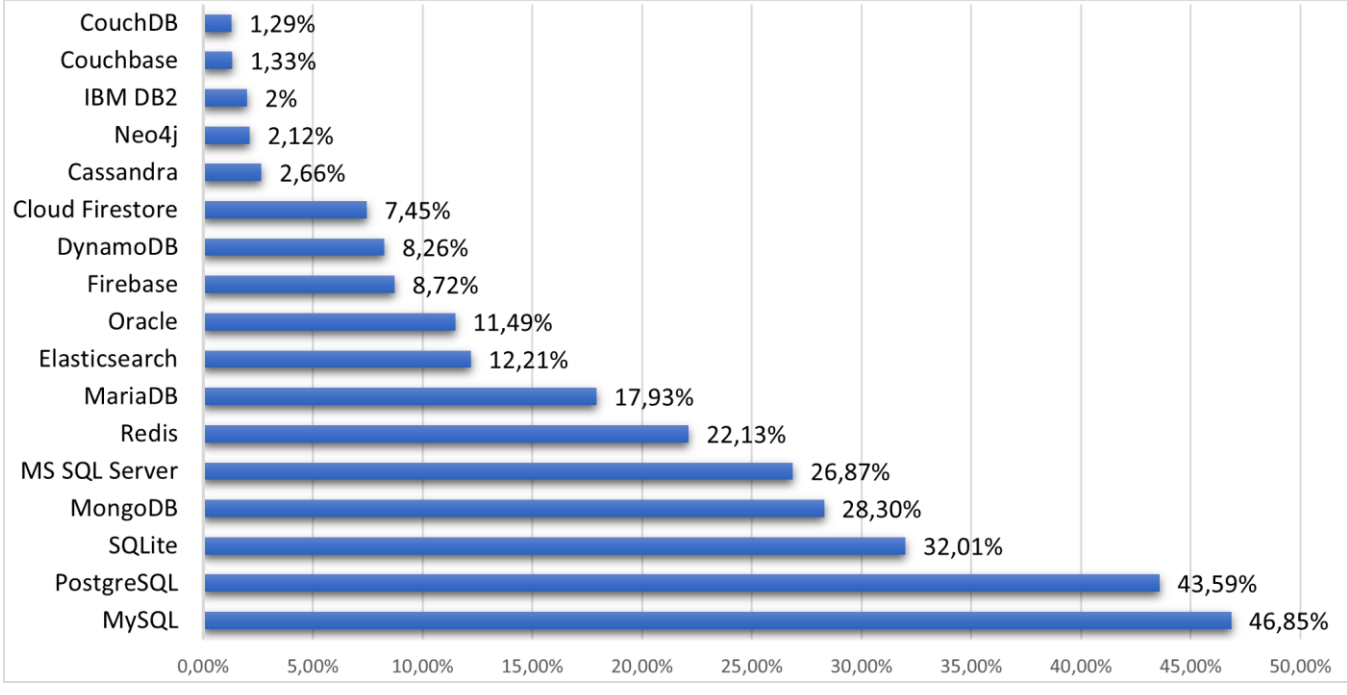


**Fig 1.**Survey of the most popular database systems (May-22)[1]

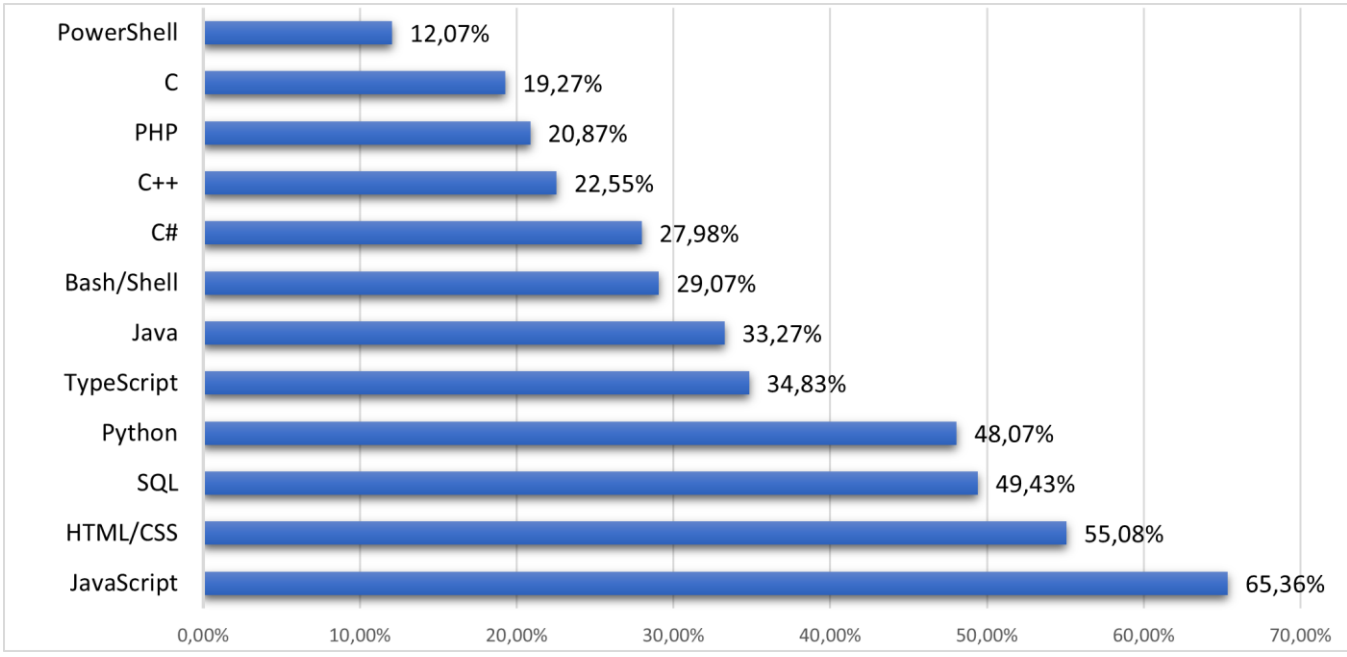


**Fig 2.** Survey of the most popular programming languages (May-22)[1]

Figure 2. as can be seen in 'Python', Python has become one of the most popular programming languages.
We can attribute this to the following features of the Python programming language.

- Simple and Readable Syntax: Python's simple and organized syntax makes the code easy to understand.
- High Level Python is a high-level programming language, meaning that it is user- friendly and does not require dealing with complex details.[11]
- Various Modules and Libraries: Python has a very rich standard library and a large ecosystem of third-party libraries that can be used in various domains.[13]
- Portability: Python can run on many platforms (Windows, Linux, macOS) and offers flexibility in portability.[14]
- Object Oriented: Python supports the object-oriented programming paradigm, meaning it can use classes and objects to model real-world entities.[15]
- Broad Community Support: Python has a large and vibrant community of users worldwide, enabling questions to be answered quickly and solutions to be found.[15]

**Areas of Use**
Python's use cases are quite brad and include.[12]

- Web Development (Django, Flask)
- Data Science and Artificial Intelligence (NumPy, Pandas, TensorFlow, PyTorch)
- Machine Learning (Scikit-learn)
- Computer Vision (OpenCV)
- Game Development (Pygame)
- REST API and Web Framework Creation
- Python
- Network Programming
- Automation and Scripting
- Scientific Calculations
- Financial Analysis
- Web Reset (Beautiful Soup, Scrapy)

**Advantages and Disadvantages Compared to Other Programming Languages**
Advantages of Python:

- Readable and simple syntax
- Extensive library and module support
- Usability on various platforms
- Ideal for rapid prototype development
- It has a large and active community

Disadvantages of Python:

- Slow execution speed: May run slower than languages like C or C++
- Not suitable for mobile application development
- There is no strong type system, so error detection can be difficult in large- scale projects

**C# PROGRAMMING LANGUAGE**
**C# Overview**
C# is a powerful and modern programming language developed by Microsoft with an open-source development environment and is typically used for the Windows platform. C# was developed by Microsoft in the late 1990s, inspired by Java, C++ and other programming languages, and became the core language of the .NET platform. Developed by Anders Hejlsberg and his team, who also partially founded the Delphi language, the language was officially released as C# 1.0 in 2002.[16] C# 2.0 was released with Visual Studio 2005, especially with the

language's extended language features, generics, nullable types, anonymous methods, and other enhancements. C# 3.0 was released with the .NET Framework 3.5 with the Language Integrated Query (LINQ) feature, which makes data manipulation easier and more natural. C# 5.0 in 2012 and C# 7.2 in 2017 included important language improvements such as async/await, pattern matching, nullable reference types, and local functions. Since 2019, this language has continued to evolve and is now used as C# 13, which was released with the current version .NET 9.[17]
Here are some of the features that make the C# language powerful;[17]

- Object Oriented Programming (OOP): C# is based on the object-oriented programming paradigm. This refers to an approach where data and functions are organized into classes, and interaction between these classes is enabled. OOP principles increase code reusability, maintainability, and extensibility.
- Extensive Library Support: C# comes with a rich standard library. This library can be used to perform a variety of tasks, such as file processing, network programming, database access, GUI development and more. Also, the .NET platform has a large ecosystem of third-party libraries and tools.
- Advanced Language Features: C# is constantly evolving as a modern programming language. Features added in recent years include async/await, LINQ (Language Integrated Query), nullable reference types. These features make code more readable, secure, and performant.
- Comprehensive Development Tools: Comprehensive integrated development environments (IDEs) such as Microsoft Visual Studio are available for C# development. These IDEs facilitate development processes such as writing, debugging, testing, and deploying code.

As seen in the
Fig, C# has become one of the most popular programming languages among the most popular programming languages.

**Areas of Use**
C# is a powerful programming language with broad industrial and academic support. Its high performance, reliability, and broad library support allow developers to build complex and scalable applications on a variety of platforms.[17]

- Web Development and Web Services: C# is used in conjunction with technologies such as ASP.NET and ASP.NET Core to develop web applications and websites. These technologies offer a powerful infrastructure and extensive library support.
- Mobile Application Development: C# is used in conjunction with frameworks such as Xamarin to develop mobile applications. This enables app development for Android and iOS platforms in a single code base.
- Game Development: C# is used in conjunction with the Unity game engine for game development. Since C# is powerful, the famous game development platform, Unity, is preferred by many indie and professional game studios.
- Desktop and Console Applications: C# is used in conjunction with technologies such as Windows Presentation Foundation (WPF) and Windows Forms to develop desktop and console applications. This enables the creation of user-interface-oriented applications for the Windows operating system.
- Data Science and Analytics: C# is used to perform various data science and analytics operations on the .NET platform. In particular, programs written in C# can be used to process and analyze large data sets.
- Financial Applications: C# is widely used in the development of financial applications. In particular, it enables the creation of reliable and performant applications in the banking and finance sectors.
- Software Agents and Systems: C# is used in the development of automation tools, system tools, management tools, and other software tools. This enables the creation of easy-to-use and powerful tools in many different industries and business areas.

**Advantages and Disadvantages Compared to Other Programming Languages**
Advantages

- Integration with .NET Platform
- Object Oriented Programming (OOP) Support
- Extensive Library Support
- Advanced IDE Support

Disadvantages:

- Platform Dependency
- Learning Curve
- Strict Dependency
- Performance Issues

**TEST ENVIRONMENT AND METADOLOGY**
**Data Preparation**
The data that to be loaded into the database with SQL DML operations is prepared in accordance with the following guidelines:

- The data was saved in JSON format as 20,000 randomly generated person data with 10 columns including ItemID, ItemGuid, Ad, Soyad, Doğum Tarihi, Adres, Telefon, LisansID, Numara, Özgeçmiş.
- Columns are created in int, uniqueidentifier, datetime, decimal, and nvarchar data types, which are commonly used in every database.
- The data is generated using a library called Faker, which has both a C# and Python library for generating random data.[18] The library and the same data were taken from the same file and used in both programs.

The generation of the data was done with the following code snippets.

```
List<Kisi> kisiler;
 public void Olustur()
 {
    string sayi, path = string.Empty;
    Console.WriteLine("Lütfen oluşturulacak kişi sayısı girin.");
    sayi = Console.ReadLine();
    int ksayi;
    if (int.TryParse(sayi, out ksayi))
    {
        Console.WriteLine("Lütfen kişilerin kayıt edileceği dosya yolunu girin.");
        path = Console.ReadLine();
        int i = 1;
        kisiler = new List<Kisi>();
        while (i <= ksayi)
        {
           Kisi data = new Kisi
           {
              ItemID = i,
              ItemGuid = Guid.NewGuid(),
              Ad = Faker.Boolean.Random() == true ? Faker.Name.First() :     Faker.Name.First() + " " + Faker.Name.Middle(),
              Soyad = Faker.Name.Last(),
              Adres = Faker.Address.StreetAddress(Faker.Boolean.Random()) + " / " + Faker.Address.City() + " - " + Faker.Address.Country(),
              DogumTarihi = Faker.Identification.DateOfBirth(),
              Numara = Faker.RandomNumber.Next(10000, 500000),
              LisansID = Faker.Identification.SocialSecurityNumber(),
              Ozgecmis = Faker.Lorem.Sentence(Faker.RandomNumber.Next(80, 150)),
              Telefon = Faker.Phone.Number()
           };
           kisiler.Add(data);
           i++;

        }
        JSONOlustur(kisiler, path);
     }
     Console.WriteLine(string.Format("{0} adet kişi, {1} dosyasına başarıyla kaydedildi. Çıkmak için Enter...",sayi,path));
     Console.ReadKey();
  }
```

**Code Snippet 1.** The code that generates the test data

```
public class Kisi
{
   public int ItemID { get; set; }
   public Guid ItemGuid { get; set; }
   public string Ad { get; set; }
   public string Soyad { get; set; }
   public DateTime DogumTarihi { get; set; }
   public string Adres { get; set; }
   public string Telefon { get; set; }
    public string LisansID { get; set; }
    public decimal Numara { get; set; }
    public string Ozgecmis { get; set; }

    public override string ToString()
    {
        return string.Format("ItemID:{0}\nItemGuid:{1}\nAd:{2}\nSoyad:{3}\n Adres:{4}}\nDoğum Tarihi:{5}\nTelefon:{6}\n
LisansID:{7}\nNumara:{8}\n Ozgeçmiş:{9}", ItemID, ItemGuid, Ad, Soyad, Adres,DogumTarihi.ToShortDateString()
,Telefon,LisansID,Numara,Ozgecmis );
    }
}
```

**Code Snippet 2.** Person class code

An example of person data generated using Code Snippet 1 is shown below in JSON format.

```
{
  "ItemID": 1,
  "ItemGuid": "3a3b5691-f464-43d8-a56b-c8b2bf88d402",
  "Ad": "Hassan",
  "Soyad": "Auer",
  "DogumTarihi": "1985-09-11T00:00:00Z",
  "Adres": "6603 Judd Avenue Apt. 289 / South Ken - Guinea",
  "Telefon": "(883)857-2727 x9877",
  "LisansID": "157-78-1606",
  "Numara": 353583.0,
  "Ozgecmis": "Doloremque suscipit dicta qui consectetur non assumenda quos molestias voluptatem adipisci aspernatur aliquid sint quia ab ipsum corporis quo consequatur dolores natus dolor quae et consequatur et earum consequatur delectus odit praesentium exercitationem quia sunt culpa aperiam molestias id modi aut labore exercitationem voluptas illum suscipit dolores dolor sapiente animi optio laborum earum aut dignissimos et saepe nihil ex deserunt quaerat nesciunt et est at totam odio omnis et sed aut natus earum qui ad et esse rerum temporibus labore et nihil est velit odit nam non dolores eveniet deserunt et eligendi autem praesentium non laborum placeat ex consequatur asperiores odit quisquam sed reprehenderit atque fuga laborum aut doloribus sed sit voluptatem omnis et doloribus aut accusamus excepturi quidem totam in eaque omnis non non sit aut fugit asperiores veniam molestiae consequuntur aut blanditiis aut eaque deserunt cum eos saepe odit harum impedit."
},
```

**Code Snippet 3.** Sample person data in JSON format

A total of 20,000 non-real-person data was created using Code Snippet 1 and this data was saved as a file in JSON format. This file was read into the prepared C# and Python programs and uploaded into the database, and tests were performed.

**Preparation of Database**

The database to perform SQL DML operations was created using Code Snippet 4 created from on the SQL Server 2014 program installed on the same computer where the test programs run. The database was named KisilerVT, and has a single table named TB_Kisiler. The newly created database file is 5.00 MB (5,242,880 bytes), and the log file is 2.00 MB (2,097,152 bytes).

```
USE [master]
GO
CREATE DATABASE [KisilerVT]
 ON  PRIMARY
( NAME = N'KisilerVT', FILENAME = N'C:\Test\KisilerVT.mdf' , SIZE = 5120KB , MAXSIZE = UNLIMITED, FILEGROWTH = 1024KB )
 LOG ON
( NAME = N'KisilerVT_log', FILENAME = N'C:\Test\KisilerVT_log.ldf' , SIZE = 2048KB , MAXSIZE = 2048GB , FILEGROWTH = 10%)
GO
```

**Code Snippet 4.** SQL code for creating a KisilerVT database

```
USE [KisilerVT]
GO
SET ANSI_NULLS ON
GO
SET QUOTED_IDENTIFIER ON
GO
CREATE TABLE [dbo].[TB_Kisiler](
	[ItemID] [int] NOT NULL,
	[ItemGuid] [uniqueidentifier] NOT NULL,
	[Ad] [nvarchar](50) NOT NULL,
	[Soyad] [nvarchar](50) NOT NULL,
	[Adres] [nvarchar](255) NOT NULL,
	[DogumTarihi] [datetime] NOT NULL,
	[Telefon] [nvarchar](50) NOT NULL,
	[Numara] [decimal](6, 0) NOT NULL,
	[LisansID] [nvarchar](50) NOT NULL,
	[Ozgecmis] [nvarchar](max) NOT NULL
) ON [PRIMARY] TEXTIMAGE_ON [PRIMARY]
GO
```

**Code Snippet 5.** SQL code to create TB_Kisiler table

To create the KisilerVT database, and the TB_Kisiler table; Code Snippet 4 and Code Snippet 5 were used. When the testing of a language is complete, the database and table were dropped and re-created for another run using these codes.

**Determination of Benchmark Conditions and Scenarios**

**Comparison Conditions**

The conditions for comparison are as follows:

- Both programs were run on the same computer and through the Console Application in order to provide the same conditions and to get rid of the advantages and disadvantages of the IDE.
- The same data file was used in both programs.
- The database and table structure were rebuilt in both programming languages prior to testing.
- SQL DML operations are performed in the same way and in the same way from a software point of view, the difference being the two programming languages and the amount of code that needs to be written.
- In each comparison scenario, the same process was repeated three times, and the average of these three times was taken as the processing time.

**Comparison Scenarios**

As known, SQL DML operations cover 4 types of operations: INSERT, SELECT, UPDATE and DELETE.[2] Comparison scenarios were defined for each function. Random ItemID values were randomly selected between 1 and 20000 (11680) and saved as a txt file to be used in both programs.

The comparison for the INSERT function was made by adding new records through the following 3 different scenarios. These are;

- Inserting 20000 records by sending a separate query for each record,
- Inserting 20000 records by sending a query for every 100 records,
- Inserting 20000 records by sending a query for every 1000 records.

It was determined as adding it to the database and transaction times were recorded.

The comparison for the SELECT function was done by calling the records through the following 3 different scenarios. These are:

- Calling 20000 records in a single query and adding them to the list structure as Person object,
- Calling 5000 records in the order in which they are in the TB_Kisiler table, that is, in a single query with the TOP structure and adding them to the list structure as a Person object,
- Calling 11680 records in a single query with the WHERE structure over pre-selected ItemIDs and adding them to the list structure as Person objects.

It was determined as calling from the database and transaction times were recorded.

The comparison for the UPDATE function was made by updating the "Ozgecmis" field with the longest data over 3 scenarios with a new Ozgecmis value defined with the same value in both programs. These are;

- Updating the "Ozgecmis" field of 20000 records in a single query,
- Updating 5000 records in the TB_Kisiler table, that is, updating the "Ozgecmis" field in a single query with the TOP structure
- Updating the "Ozgecmis" field in a single query with the WHERE structure over pre-selected ItemIDs of 11680 records,

Updates were made in the database, and transaction times were recorded.

The comparison for the DELETE function was made by deleting records in the following three different scenarios. These are;

- Deleting 20000 records from the database in a single query,
- Deleting 5000 records from the database in the order they are in the TB_Kisiler table, that is, in a single query with the TOP structure,
- Deleting 11680 records from the database in a single query with WHERE structure over pre-selected ItemIDs,

Deletion was made from the database and transaction times were recorded.

**Preparatıon of Python and C# Programs**

**Python Program Codes**

The Python program was prepared as 2 different files. These are; main.py file containing the start function and main functions, and kisi.py file containing the Kisi class.

**C# Program Codes**

The C# program was prepared as 2 different files. These are: Program.cs file containing the start function and main functions, and Kisi.cs file containing the Kisi class.

## COMPARISON OF PERFORMANCE AND CODE WRITING

**Performance Comparison**

**C# Performance Tests and Results**

After all tests, the database file size was 81.0 MB (84,934,656 bytes), and the log file size was 214 MB (224,526,336 bytes), even though there was no data in the database.

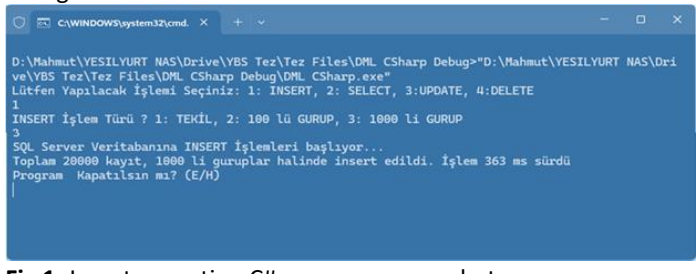


**Fig 1.** Insert operation C# program screenshot

**Table 1.** C# Program Insert operation times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 3976 | 811 | 363 |
| **2. Operation** | 3797 | 792 | 323 |
| **3. Operation** | 3802 | 789 | 340 |
| **Average** | **3858** | **797** | **342** |

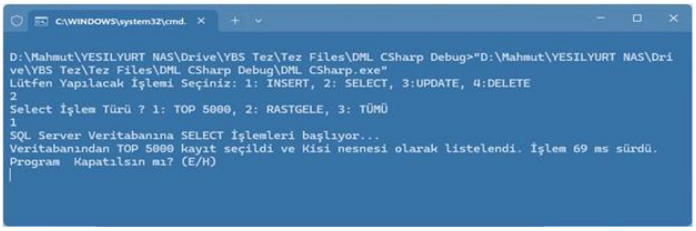


**Fig 2.** Select process C# program screenshot

**Table 2.** C# Program Select processing times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 97 | 884 | 284 |
| **2. Operation** | 69 | 885 | 264 |
| **3. Operation** | 75 | 893 | 280 |
| **Average** | **80** | **887** | **276** |

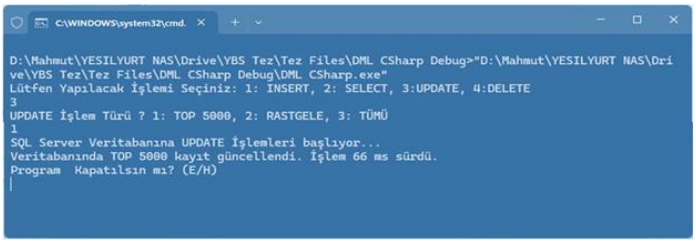


**Fig 3.** Update process C# program screenshot

**Table 3.** C# Program Update processing times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 66 | 7105 | 265 |
| **2. Operation** | 62 | 7128 | 269 |
| **3. Operation** | 63 | 7132 | 258 |
| **Average** | **64** | **7122** | **264** |


**Fig 4.** Delete process C# program screenshot

**Table 4.** C# Program Delete processing times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 597 | 7017 | 45 |
| **2. Operation** | 500 | 6950 | 42 |
| **3. Operation** | 496 | 6998 | 45 |
| **Average** | **531** | **6988** | **44** |

**Python Performance Tests and Results**

After all tests, the database file size was 94.0 MB (98,566,144 bytes), while the log file size was 247 MB (258,994,112 bytes), even though there was no data in the database.

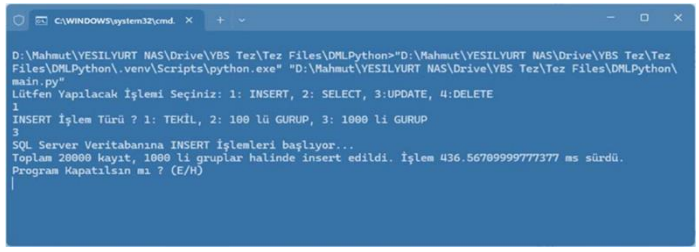


**Fig 5.** Insert process Python program screenshot

**Table 5.** C# Program Select processing times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 3358 | 871 | 436 |
| **2. Operation** | 3139 | 879 | 443 |
| **3. Operation** | 3176 | 878 | 422 |
| **Average** | **3224** | **876** | **434** |

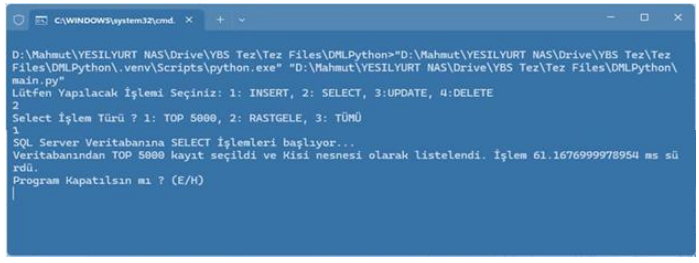


**Fig 6.** Select process Python program screenshot

**Table 6.** C# Program Select processing times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 61 | 935 | 237 |
| **2. Operation** | 56 | 922 | 235 |
| **3. Operation** | 57 | 917 | 240 |
| **Average** | **56** | **925** | **237** |

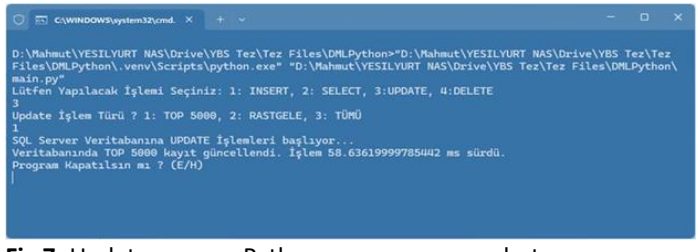


**Fig 7.** Update process Python program screenshot

**Table 7.** C# Program Select processing times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 58 | 6851 | 290 |
| **2. Operation** | 55 | 7245 | 270 |
| **3. Operation** | 57 | 6886 | 224 |
| **Average** | **57** | **6994** | **261** |

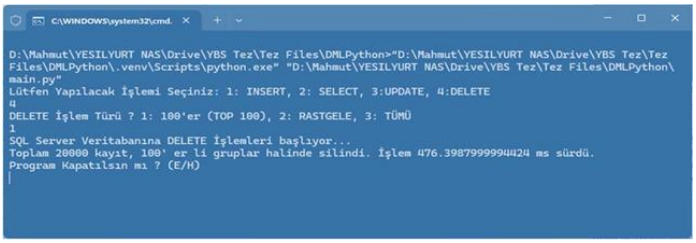


**Fig 8.** Delete process Python program screenshot

**Table 8.** C# Program Select processing times test results (ms)

| | Scenario 1 | Scenario 2 | Scenario 3 |
|---|---|---|---|
| **1. Operation** | 538 | 6778 | 43 |
| **2. Operation** | 490 | 7038 | 44 |
| **3. Operation** | 476 | 6834 | 37 |
| **Average** | **501** | **6883** | **41** |

**Process Performance Comparison**

**Table 9.** Comparison of both programming languages on Average Processing times

| Process | C# Average Transaction Duration (ms) | Python Average Transaction Duration (ms) | Difference Rate (%) | Conclusion |
|---|---|---|---|---|
| **Insert - S1** | 3858 | 3224 | 19,66% | Python |
| **Insert - S2** | 797 | 876 | 9,87% | C# |
| **Insert - S3** | 342 | 434 | 26,80% | C# |
| **Select - S1** | 80 | 58 | 38,51% | Python |
| **Select - S2** | 887 | 925 | 4,21% | C# |
| **Select - S3** | 276 | 237 | 16,29% | Python |
| **Update - S1** | 64 | 57 | 12,35% | Python |
| **Update - S2** | 7122 | 6994 | 1,83% | Python |
| **Update - S3** | 264 | 261 | 1,02% | Python |
| **Delete - S1** | 531 | 501 | 5,92% | Python |
| **Delete - S2** | 6988 | 6883 | 1,53% | Python |
| **Delete - S3** | 44 | 41 | 6,45% | Python |

**Code Writing Comparison**

**Comparison of Code Size and Readability**

C# is a statically typed programming language, which means that when defining a variable, its type must be specified. For example, a variable is declared as int a = 5;. Python, on the other hand, is a dynamically typed language. Here, no need to specify the type of variables in advance. A variable can simply be defined like a = 5;. This flexibility gives Python an advantage in rapid prototyping and code writing. However, in terms of safe code writing, statically typed languages are safer. Also, on the code readability side, this makes code readability difficult, and in large projects, it can be difficult to understand the code when looking at older code. At this point, statically typed languages are more advantageous.

The Python program code has a total of 9,688 characters, 203 lines, 891 words, and at a size of 9.46 KB (9,688 bytes).

The C# program codes totaled 16,566 characters, 330 lines, 1770 words, and at a size of 16.1 KB (16,556 bytes).

**Table 10.** Comparison of both programming languages on code parameters

| Parameter | C# | Python | Ratio | Conclusion |
|---|---|---|---|---|
| **Character** | 16566 | 9688 | 71,0% | Python |
| **Line** | 330 | 203 | 62,6% | Python |
| **Word** | 1770 | 891 | 98,7% | Python |
| **Size (KB)** | 16,1 | 9,46 | 70,2% | Python |

**Debugging and Optimization Comparison**

Although C# and Python are different software languages, they both have powerful tools for debugging and code optimization. C# offers debugging and optimization tools such as Visual Studio Debugger, Debug and Trace output generation classes "Debug.WriteLine()" and "Trace.WriteLine()" functions, real-time debug and break- point support, exception management with try-cache blocks, and profiling tools. Python, on the other hand, offers powerful debugging and code optimization tools with its powerful standard library and specially designed plugins for debugging. Some of these include the pdb and pdb++ debugger classes, real-time debug and break-point support, exception handling with try-cache blocks, the pytest and unittest classes, and the logging class.

**CONCLUSION**

In this study, two separate computer programs were written to perform SQL Server DML operations using C# and Python, and with these programs, the content of the JSON file containing 10 columns and 20,000 rows of data was read with the program, the data was first converted into a list of objects, and the basic DML operations were implemented with the programs written in both languages over the specified process scenarios and comparisons were made in terms of both performance measurements and code writing. For performance measurements, each operation was repeated three times, and the average of these was considered the processing time.

Evaluation in terms of processing performance: A total of four operations were performed with three different scenarios, and although it seems that the Python language performed better mathematically when looking at the processing times of these 12 operations, it was observed that both programming languages did not provide a significant superiority over the other.

Evaluation in terms of code size and readability: In terms of code readability, C# can be considered a more readable language in a general context, but in the context of the programs written for this study, there was no difference between both programming languages in terms of code readability. Both in the general context and in the context of the program written within the scope of this thesis in terms of code size Table 11 when looking at the results in 'Python has a clear advantage.

Evaluation in terms of debugging and optimization: Both languages have been shown to have a high level of debugging and code optimization capabilities, so the main factor that will

determine the choice will be fitness for purpose.
As a result, it has been determined that both languages have superior features compared to each other, and that there is no definite superiority of these two languages over each other in DML operations. The choice of language should be based on the requirements of the project, the ecosystem, and the skills of the team.

**Peer-review**: Externally peer-reviewed.

**Author Contributions:** Concept – M.Y.; Design – M.Y.; Supervision – Y.Z.A., Resources – K.K.; Data Collection and/or Processing – Y.Z.A., M.Y.; Analysis and/or Interpretation – Y.Z.A., M.Y.; Literature Search – M.Y; Writing Manuscript – Y.Z.A., M.Y.; Critical Review – Y.Z.A., M.Y.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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**REFERENCES**

1. Stackoverflow.com. Stack Overflow Developer Survey 2022. Stack Overflow. https://survey.stackoverflow.co/2022#most-popular-technologies-misc-tech. Accessed May 11, 2024.
2. Setiyadi D. Database System Development Life Cycle (DSDLC) on System Libraries for Data Manipulation Language (DML) Using SQL Server 2008. *J Mantik*. 2021;5(2):1065-1066. https://www.ejournal.iocscience.org/index.php/mantik/article/view/1448/1032. Accessed May 1, 2024.
3. Hakim Lubis J, Muisa Zamzami E. Relational Database Reconstruction from SQL to Entity Relational Diagrams. *J Phys Conf Ser*. 2020;1566(1). doi:10.1088/1742-6596/1566/1/012072.
4. Codd EF. A Relational Model of Data for Large Shared Data Banks. *Commun ACM*. 1970;13(6):377-387. doi:10.1145/362384.362685.
5. Microsoft Learn. ACID Properties - Win32 apps | Microsoft Learn. https://learn.microsoft.com/en-us/windows/win32/cossdk/acid-properties. Accessed May 11, 2024.
6. Taylor P. Most popular relational DBMS 2022 | Statista. https://www.statista.com/statistics/1131568/worldwide-popularity-ranking-relational-database-management-systems/. Accessed May 11, 2024.
7. Azure Microsoft. NoSQL Database - What is NoSQL? https://azure.microsoft.com/tr-tr/resources/cloud-computing-dictionary/what-is-nosql-database/. Accessed May 11, 2024.
8. Amazon.com. NoSQL Nedir? | Non-Relational Databases, Flexible Schema Data Models | AWS. https://aws.amazon.com/tr/nosql/. Accessed May 11, 2024.
9. Bektaş G. The Importance of Data Management and the Role of NoSQL Databases: ACID, CAP, and BASE Concepts and Principles. https://medium.com/@bektasguner772/the-importance-of-data-management-and-the-role-of-nosql-databases-acid-cap-and-base-concepts-and-principles-e7c572742098. Accessed May 11, 2024.
10. Innuy. List of Best and Most Popular NoSQL Database 2022. https://www.innuy.com/blog/list-of-best-and-most-popular-nosql-database-2022/. Accessed May 11, 2024.
11. Saralıoğlu E. *Python Programlama Dili Kullanılarak Uzaktan Algılama Amaçlı Arayüz Tasarımı* [master's thesis]. Karadeniz Technical University; 2015.
12. Ayvaz U, Çoban A, Gürüler H, Peker M. Python Dilinin Öznitelikleri, Programlama Eğitiminde ve Yazılım Dünyasındaki Yeri. *Akad Bilişim*. 2016;1-6.
13. Summerfield M. *Programming in Python 3: A Complete Introduction to the Python Language*. 2nd ed. http://ifile.it/qx63lw/ebooksclub.org__Programming_in_Python_3__A_Complete_Introduction_to_the_Python_Language__2nd_Edition_.pdf. Accessed May 9, 2024.
14. Scarlett R. Why Python keeps growing, explained - The GitHub Blog. https://github.blog/2023-03-02-why-python-keeps-growing-explained/. Accessed May 11, 2024.
15. Python.org. Python 3 Documentation. https://docs.python.org/. Accessed May 11, 2024.
16. Kimmel P. *Advanced C# Programming*. McGraw-Hill/Osborne; 2002.
17. Microsoft Learn. C# Guide .NET managed language | Microsoft Learn. https://learn.microsoft.com/en-us/dotnet/csharp/. Accessed May 12, 2024.
18. Riches O. GitHub - oriches/faker-cs: C# port of the Ruby Faker gem. https://github.com/oriches/faker-cs. Accessed May 11, 2024.

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# Current perspectives on Multiple Sclerosis

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## ABSTRACT

Multiple Sclerosis (MS) is a chronic, autoimmune disease that affects the central nervous system. It is characterized by inflammation, demyelination and axonal loss, and is typically manifested by relapses and remissions. It is the most prevalent neurological disorder worldwide, with a significant prevalence in many countries. It is the leading cause of non-traumatic neurological impairment in young adults. Although the etiology is not fully understood, genetic predisposition, environmental factors (exposure to inadequate sunlight and/or inadequate dietary intake of vitamin D, Epstein-Barr virus infection, etc.) Furthermore, an individual's lifestyle, including obesity, smoking, and other factors, plays a significant role in the development of the disease. The clinical subtypes of MS, as defined in 2013, are classified into four categories: The four main clinical subtypes of MS are: Isolated Syndrome, Relapsing-Remitting MS, Primary Progressive MS and Secondary Progressive MS. The clinical subtypes of MS are further subdivided according to the activity and progression of the disease.

MS is a heterogenous disease, with lesions affecting multiple systems. The most common clinical manifestations include fatigue, blurred vision, and ocular pain (optic neuritis), as well as weakness and sensory changes in specific body regions, such as the face, arms, and legs. Furthermore, the patient presented with symptoms including balance impairment, vertigo, memory and cognitive difficulties, and bladder control issues.

Although there is currently no cure for MS, existing treatments focus on alleviating acute attacks, improving symptoms, and reducing the impact of the disease through biological therapies. Modifying therapies for the disease (e.g., interferons, glatiramer acetate, dimethyl fumarate, teriflunomide, fingolimod, ocrelizumab, natalizumab, etc.) These drugs, which reduce the frequency of clinical attacks and slow the progression of the disease, also reduce the activity of MRI lesions, making them an important component of MS treatment. They are effective due to their diverse mechanisms of action, administration routes, and dosages.

**Keywords:** Attack, Demyelination, Inflammation, Multiple Sclerosis

## INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory and neurodegenerative disease caused by autoimmune reactions that cause the central nervous system (CNS) to progressively disappear.[1,2] It was first described in 1868 by the French neurologist and pathologist Jean-Martin Charcot as a new disease of the nervous system.[3] MS is a disease that usually progresses with attacks, and myelin sheath and axon degeneration due to attacks may cause physical sequelae in patients, and a progressive disability may occur in patients with the accumulation of sequelae.[4]

MS is one of the most common diseases that cause neurological disorders worldwide and is the leading cause of non-traumatic neurological impairment among young adults in many countries.[5] According to the Atlas of MS, published by the International Federation of MS (MSIF), there are 2.9 million MS patients worldwide.[6] Although the onset of symptoms typically occurs in young adults between the ages of 20 and 30, women are about three times more likely to develop MS than men.[7] MS is not just a disease that affects adults. According to data from 55 countries reporting to MSIF, there are 30,000 children under the age of 18 living with MS worldwide. [6]

The prevalence and incidence of MS varies geographically. The prevalence of the disease is higher in America (100,000 to 111 people) and in Europe (100000 to 137 people), while it is lower in Africa and the Western Pacific (100,000 people to 5 people) (Figure 1).[6] Prevalence studies conducted in the past have shown that the incidence of MS is related to latitude, with the number of MS cases decreasing closer to the equator and the incidence increasing as the latitude increases (closer to the North/South poles). The slendering-related increase in MS prevalence is explained by the assumption that people living at higher geographical altitudes may have been less exposed to sunlight and thus have lower levels of vitamin D.[8]

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Although the etiology of MS is not clear, it is known that autoimmunity, impaired immune system, genetic and environmental factors (vitamin D levels, obesity, smoking and Epstein Barr virus (EBV) infection) are influential in the onset of the disease.[9]

The pathogenesis of MS is caused by infiltration of the focal immune cell and cytokine, leading to inflammation of white and gray matter tissues.[10] Oligodendrocytes are damaged and demyellinized in the early stages of the disease due to acute inflammation. However, irreversible axonal loss is initially less pronounced and tends to increase as the disease progresses. Typical MS lesions are identified in the pons, spinal cord and periventricular region but can be seen in any region of CNS. Other common localizations include the entire cerebral cortex, cerebral, cortical and jukstakortical regions.[11] The symptomatology of MS is diverse, heterogeneous, and may vary depending on the anatomical distribution of the lesions. Symptoms of MS include fatigue, blurred vision and eye pain (optic neuritis), weakness or sensory changes in certain parts of the body, such as face, arms or legs, trouble balancing, dizziness, memory and thinking impairment, and problems with bladder control. MS patients are also at high risk of depression and anxiety.[7]

The diagnosis of MS is based on evidence of CNS lesions spreading in time and space, as well as neurological symptoms and findings.[12]

The primary goal of MS treatment is to prevent permanent disability by reducing the inflammatory response and resulting neuroaxonal damage. One of the most important factors affecting the success of treatment is the correct and early diagnosis of the right treatment at the right time.[13]

This article aims to provide information about the increasing prevalence of MS worldwide and the effects of the treatment options used.

**MS**

MS is a chronic, autoimmune, neurodegenerative disease characterized by inflammation, demyelination and axon loss of CNS.[14] The term "multiple sclerosis" is derived from the Greek word "sklerosis", which refers to the formation of hardened areas in the lesions present in the "multiple" sections of the CNS.[3] The definitive definition of MS was first made in 1868 by Jean-Martin Charcot.[15] MS is a disease that is often followed by attacks and remissions, which progresses over the years.[16] These seizures or progressive degenerative processes can reduce the quality of life of patients and cause permanent impairment.[17] One or more neurological deficits consistent with an acute inflammatory demyelinating process lasting at least 24 hours in the absence of fever and infection are defined as an attack. Attacks are directly related to the diagnosis, prognosis and treatment of MS, as well as the main determinant of subtypes of MS.[18]

**Epidemiology**

MS is one of the most common autoimmune inflammatory diseases of CNS.[19] According to MSIF's country-level MS atlas, there are 2.3 million MS patients worldwide in 2013, 2.8 million in 2020 and 2.9 million in 2023 (Figure 1).[6] According to a report by MSIF, the global median prevalence of MS has risen to 33/100,000 in 2013, from 30 per 100,000 in 2008.[20] According to 2020 data, global median prevalence is expected to reach 35.9 per 100,000.[21] According to data from 81 countries, the number of incidents worldwide is 2.1 per 100,000 per year. This is equivalent to one person in the world being diagnosed with MS every 5 minutes.[6]

Increased epidemiological studies have shown an increased prevalence of MS in many parts of the world. This rise is interpreted by two different approaches. The first is a false rise caused by easier diagnostic methods due to the spread of magnetic resonance imaging (MRI) and the frequent change of diagnostic criteria; the second approach is that it is actually a real rise and that the disease affects more and more people. The second approach involves epigenetic changes and environmental factors.[22,23] Many epidemiological studies in the past have shown that the prevalence and incidence of MS increases as they move away from the equator. This suggests that the environmental factors caused by the differences in the geographical characteristics of the places where individuals live have an effect on the disease.[24] The prevalence of the disease is high in America and Europe, while it is low in Africa and the Western Pacific. The highest prevalence rates of MS in Europe are in Germany, Italy and the Scandinavian countries.[6]

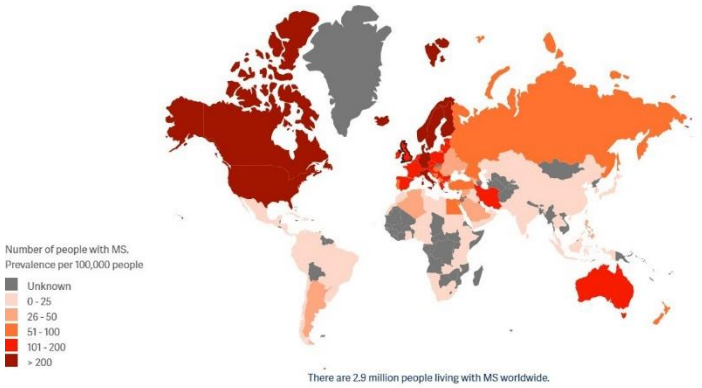


**Fig. 1.** Country-specific prevalence of MS on Earth. [6]

MS is a disease that usually affects young adults. Often, the symptoms begin between the ages of 20 and 30, but there are also patients diagnosed with MS in childhood or older years.[7,25] Pediatric MS (POMS) is defined as MS that begins before the age of 18. POMS accounts for 2% to 10% of total MS cases, and the highest incidence rate is between the ages of 13 and 16.[26–29] A systematic survey of the paediatric population, covering the years 1965-2018, found that the overall incidence ranged from 0.05 to 2.85 per 100,000 children, and the overall prevalence from 0.69 to 26.92 per 100.000 children.[30] In the pre-adolescent paediatric population, the incidence of MS observation is almost equal in girls and boys, while post adolescent girls are more likely to be affected.[26–29]

**Etiology**

The genetic predisposition, viral infections, environmental factors, and the individual's immune system are believed to be

influential in the development of MS, although the etiology is unknown.[31] Environmental factors include vitamin D deficiency, smoking, childhood obesity, non-specific infections (*Chlamydia* bacteria, etc.), fever and injuries.[9,32–35] Smokers have about twice the risk of developing MS than non-smokers.[36] Some important findings suggest that smoking causes the release of inflammatory cytokines, which are strongly linked to increased risk of neurodegenerative diseases such as MS.[37]

**Risk factors**

Lifestyle, genetic and environmental factors contribute significantly to the risk of developing the disease.[38] Genetic predisposition explains only part of the disease risk, but lifestyle and environmental factors are key factors that contribute to the risk of MS. High blood pressure, female sex, adolescent obesity, smoking, insufficient sun exposure and/or low vitamin D levels due to dietary intake, EBV infection, and organic solvents are some of the risk factors associated with MS. All of the risk factors described for MS can affect adaptive and/or congenital immunity modulated by MS risk alels. Unlike genetic risk factors, many environmental and lifestyle factors can be modified to provide prevention potential for those at the highest risk, especially the relatives of people with MS.[39]

**Genetic Factors**

The clustering of MS in families and the presence of genes predisposed to MS support the relationship between MS and genetics.[40] Many genes contribute cumulatively to disease risk and disease behavior, and these related genes and alleles vary from patient to patient.[41] Genes in the HLA region are the strongest genetic risk factors for MS.[42–45] HLA-DRB1 * 15: 01, HLA - DRB1* 13:03, HLA (DRB1) * 03:01, HLA "DRB" * 08:01 and HLA 'DQB' * 03:02 are classified as a risk factor for MS, while HLA's class I algae are preservative, HLA A * 02:01, hLA-B * 44:02, hla' - B * 38:01 and hla-b * 55:01.[45] HLA-DRB1 * 15: 01 allele has the strongest association with MS risk among the genetic risk factors, and has been associated with MS susceptibility in almost all populations studied.[46]

**Exposure to Infectious Agents**

Many bacteria and viruses associated with the development of MS have been identified. Bacteria that may play a role in the development of the disease include *Chlamydia pneumoniae, Porphyromonas gingivalis*, *Bacteroides fragilis* and *Staphylococcus aureus*.[47] Many persistent and chronic viral infections, primarily of the Herpesviridae family, have been considered as potential risk factors for MS. Although serological responses to these viruses have been used to assess the risk of MS, the direct mechanism of the pathogen's contribution to the development of the disease is not known. Accordingly, common theories include direct infection of the surrounding CNS tissue or oligodendrocytes, irregularity of immune tolerance mechanisms, and the possibility of molecular imitation between pathogens or autoantigens.[48] The most common viral risk factors for MS include human herpes virus 6 (HHV-6), Epstein-Barr virus (EBV), varicella-zoster virus (VZV) and human endogenous retroviruses.[47] Self antigens of HHV-6 and EBV viruses show molecular myelin similarity. This similarity suggests that they initiate autoimmune reactions with impaired immune tolerance to myelin proteins.[9]

**Vitamin D**

There is significant evidence that vitamin D deficiency, which has a significant impact on the development and function of CNS, may be a significant risk factor for the development of MS, especially in children.[49] Decreased levels of vitamin D are considered not only a risk factor for MS, but also a possible factor in the severity and incidence of the disease.[50] A study by Munger *et al.*[51] has shown that increased levels of vitamin D, especially before the age of 20, are associated with a decrease in MS in the later stages of life.[52,53] Historically, it has been suggested that low levels of vitamin D can support an irregular and/or hyperactive immune system that leads to CNS inflammation, making this system vulnerable to inflammations.[49] The polymorphisms in the genes DBP (vitamin D binding protein), VDR (vitamine D receptor), CYP27B1 and CYP24A1 may appear to be effective in MS sensitivity, as they play a role in both vitamin D levels and functions.[46]

**Smoking**

Smoking is one of the most prominent environmental risk factors associated with the onset and progression of MS in genetically sensitive individuals.[9,54] Observational studies have shown an increased risk of MS by about 50% compared to non-smokers.[55–59] These studies also show that there is a clear dose-response relationship between the cumulative dose of smoking and the risk of MS, and that both the duration and intensity of smoke are independent risk factors.[60,61] Also, passive smoking has been associated with an increased risk of MS.[62] Smoking is not only associated with an increased risk of MS, but also with the risk of developing neutralizing antibodies against biological substances such as interferon-β (IFN-β) and natalizumab used in the treatment of MS.[63,64] Several hypotheses have been proposed to explain the increased risk of MS in smokers, including effects on the immune and cardiovascular systems, increased frequency of respiratory infections, and neurotoxic effects of cigarette smoke metabolites.[65]

Cigarettes contain high amounts of free radicals. Oxidative stress caused by these free radicals has been to cause mutations in genetic material and to play a role in various neurodegenerative disorders such as MS.[66,67] Cigarette smoke has an effect on the immune system at the cellular level and causes the release of cytokines that cause inflammation.[66–68] The level of pro-inflammatory cytokines such as C-reactive protein, fibrinogen and other inflammatory markers, as well as interleukin (IL)-1B, IL-6, IL-23, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, is higher among smokers.[66,68] High levels of pro-inflammatory cytokines can contribute to permanent autoimmunity. In general, individuals with MS have higher levels of nitric oxide (NO) due to the presence of an inducible form of nitric oxide synthase (iNOS) in cells such as astrocytes and macrophages.[69] High levels of NO can lead to oligodendrosite necrosis, mitochondrial damage, axonal degeneration and ultimately impaired axonal conduction.[69,70] NO and CO (carbon monoxide) in cigarette smoke increase the inflammatory response and weaken some immune defenses, causing increased susceptibility to infections.[68,71] These changes in the immune system explain

several possible mechanisms that cause increased susceptibility to viral respiratory infections in MS patients.[66,68,71] A polyphenol-rich glycoprotein in cigarettes stimulates the proliferation of T cells and the differentiation of B cells, creating a pro-inflammatory environment.[71] In smokers, pro-inflammatory cells, such as G-protein-bound receptor 15 (GPR15) T cells are increased, and this is associated with MS.[72] Hydrogen cyanide and acrolein in cigarette smoke may also induce immunosuppression and neurodegeneration.[70,71] On the other hand, Gao and his colleagues studied the effect of nicotine and non-nicotine components in cigarette smoke on MS, using an experimental autoimmune encephalomyelitis (EAE) model in mice. Nicotine has been found to reduce the severity of EAE with reduced demyelination, increased body weight, and weakened microglial activation. This protective role of nicotine can be explained by its immunomodulatory functions.[73] Given that nicotine affects the a7 subunit of the acetylcholine receptor in immune cells by reducing the receptor activity, it stands out as an important candidate for a possible form of protection.[74]

Smoking has a significant interaction with the HLA risk genes associated with MS. Smokers who carry the HLA MS risk gene have a significantly higher risk of MS than those who do not carry this risk gene.[75] Among those carrying the genetic risk factor HLA-DRB1 15:01 but lacking protective Hla-A02, 41% of MS cases are associated with smoking.[76] Smoking has also been shown to interact with a variant of a non-HLA gene called NATI (the gene that encodes the enzyme involved in the metabolism of cigarette-related products).[36]

**Obesity**

There is an increasing number of evidence that supports the role of obesity in increased risk of MS.[77] Adolescence (~10 years) is the period when the effect of obesity on MS is strongest.[78] Adolescent obesity has been associated with an increased risk of developing MS due to both chronic inflammation and its correlation with low vitamin D levels.[51,79] A correlation was observed between body mass index (BMI >27) and relative risk of MS in obese or overweight girls.[78] Women with higher BMIs in childhood and adolescence tend to experience earlier menstruation, and this has been suggested to be associated with early onset of MS in women.[80–84] Studies found that women with a BMI of 30 kg/m$^2$ were 2.25 times more likely to develop MS when compared to women aged 18 with a normal BMI (18.5 to 21 kg/m$^2$).[85] Women's hormonal factors and childhood obesity, or X chromosome, can lead to an increase in the ratio of female to male MS, as shown by the fact that the risk of MS is higher in medium and highly obese girls, although it is not seen in males.[86] At least a few possible mechanisms have been identified that lead to an increased risk of MS in young obese individuals.[78] Obesity is associated with the onset of a low-level inflammatory condition in which increased levels of pro-inflammatory agents are produced in fatty tissue, and an increase in leptin levels, which is a medium linked to pro-inflammatory processes.[87–90] It also reduces the bioavailability of vitamin D and thus promotes pro-inflammatory processes.[91] Any of these potential mechanisms can increase the activation of adaptive auto-active immune cells, which can trigger attacks of neuro-inflammatory activity, a series of events supported by HLA gene interaction. The fact that the HLA genes encode the molecules that provide the antigen necessary for activation of T cells and interact with obesity supports the idea that obesities are prone to MS by promoting adaptive immunity related to MS neuron inflammation.[39] People with a high BMI, who carry DRB1*15:01 and do not have protective HLA-A02 have a 14 times higher risk of MS. This supports the causal role of obesity.[63] The largest genome-wide association study in which numerous MS cases were used revealed 70 different single nucleotide polymorphisms associated with BMI.[92] Similarly, a large-scale meta-analysis using Mendel randomization showed that the genetic risk score of 97 single nucleotide polymorphisms, known to be associated with higher BMI, contributed to a significant increase in POMS.[93,94]

**Geographical Latitude**

Despite the possible lack of accuracy in MS prevalence data, studies have generally confirmed that MS is more prevalent in regions with higher latitudes, and this has been associated with less sunlight intensity and lower UVB exposure in individuals.[95] Less exposure to UVB increases the risk of MS, causing less cholecalciferol production in the skin. The possible protective mechanism of UVB demonstrates its independent role in immunomodulation. UVB stimulates dentritic cells in the skin, secreting IL-10 (an anti-inflammatory cytokine thought to be protective against disease). IL-10 stimulates the local regulating T cells (Tregs) in the skin and the Tregs in the lymph nodes, and these activated Treg's perform immunomodulatory functions in CNS after entering the bloodstream.[96]

Although migration from high latitudes to low latitudes suggests a reduced risk of MS, the timing of migration has critical implications for this change.[97] Migration studies have shown that people under the age of 15 tend to embrace the risks of MS in the country where they migrate, while those older than 15 persist in their risks from the country of origin.[98] It has been discovered by Tao *et al.* that MS appears at an earlier age in populations living in areas with higher latitudes.[99]

**Salt consumption**

Studies and *in vitro* experiments clearly point to the relationship between excess salt consumption and MS risk.[100] A small study in the Argentine population found that people with high salt consumption with MS had significantly higher levels of depression and disease activity, as demonstrated by MRI, compared to those with low salt consumption.[101] *In vitro* experiments have shown that high salt consumption activates serum/glucocorticoid-regulated kinase 1, promotes T cell differentiation into pathogenic $T_H17$ cells, and mice consuming a very high salt diet develop a more severe course of EAE.[102,103] In these experiments, the amount of salt consumed by diet in humans is equivalent to >500 g of salt per day.[102]

**Intestinal microbiome**

In fecal microbiom analysis of MS patients, dysbiosis in the intestinal microbiome has been seen as a risk factor for the development of MS.[104,105] Changes in the abundance and diversity of the gut microbiome increase the permeability of the intestinal and blood-brain barrier, increasing the severity of

EAE.[106] Bacterial products, such as short-chain fatty acids, have also been associated with MS pathogenesis.[107] These are fermentation products of dietary fibre and are 95% composed of acetate, butyrate and propionate.[108,109] *In vitro* studies have shown that short-chain fatty acids such as propionate can support the polarisation of pure CD4+ T cells to Tregs.[110]

**PROTECTIVE FACTORS**

Factors such as alcohol or nicotine use, high coffee consumption and cytomegalovirus infection are associated with reduced risk of MS.[39]

**Alcohol**

A study found that Danish women with low alcohol consumption had a 44% lower risk of MS than non-drinkers.[111] In this regard, moderate alcohol consumption during adolescence has been associated with a lower risk of developing MS in the future.[112] Numerous studies involve consumption of red wine containing resveratrol.[113] Resveratrol, an antioxidant component found in grapes and fruits, is associated with preventing and delaying the onset of chronic diseases and reducing all-cause mortality.[111,114] Therefore, resveratrol has been tested in multiple rodent models of EAE MS and has shown increased remyelination, induction of blood-brain barrier repair and a decrease in proinflammatory cytokines.[115] Another potential mechanism of action of resveratrol is that activated T cells interact with aryl hydrocarbon and estrogen receptors, thereby inducing apoptosis or promoting phenotype change to the regulator Th17. This change reduces the overall serum levels of pro-inflammatory IFN-γ and IL12 cytokines.[116]

**Coffee Consumption**

The relationship between coffee consumption and MS risk has been investigated in two independent population case control studies. Those who consumption of high amounts of coffee (> 900 ml per day) had a 30% reduction in the risk of MS. Coffee contains a large number of biologically active substances, including caffeine, which stimulates CNS.[117] Caffeine, which has an antagonizing effect on the adenosine receptors found in neurons and glial cells, also binds to macrophage adenosin receptors, creating a polarizing shift from the pro-inflammatory phenotype to the anti-inflammatory phenotipe. Adenosine A1 receptors are also found in peripheral blood mononuclear cells (PBMCs), which regulate pro-inflammatory signaling for TNFα and IL-6 secretion. Blood samples from MS patients showed a decrease in serum adenosine and A1 levels, as well as fewer PBMC receptors, and also showed that TNFα was not suppressed by adenosin activation.[75]

**DIAGNOSIS**

In addition to a thorough history and physical examination, diagnostic tools necessary to diagnose MS and exclude other diagnoses include MRI, cerebrospinal fluid (CSF) analysis and evoked potential tests (Table 1).[13] MRI is the most important diagnostic and prognostic technique for the evaluation of MS (especially in the early stages of the disease) and the only technique that can interrogate the entire CNS *in vivo*.[118]

Anti-aquaporin-4 and anti-MOG antibody are clinically proven molecular biomarkers that allow differentiation between various inflammatory demyelinating diseases of the CNS.[119,120]

**Table 1.** Research on the diagnosis of MS.[13]

| **Primary Tests** |
|---|
| **1. Blood tests** (hemogram, renal and liver function tests, electrolyte levels, sedimentation, CRP, B12, folate and vitamin D, thyroid function tests, lipid panel, viral serology (anti HIV, anti HCV, HbsAg, anti-Hbs), VDRL-RPR, ANA (1/320 titer and patterns), if ANA positive ENA profile, antiphospholipid antibodies, anti-ds DNA |
| **2.MRI** (cranial, cervical and thoracal) |
| **3.CSF analyses** (CSF protein, CSF and concurrent blood glucose, CSF albumin and IgG, CSF lactate, serum albumin and IgG, CSF IgG index, CSF OCB analysis with IEF electrophoresis) |
| **4.In patients with optic neuritis: VEP and optic coherence tomography** |
| **Secondary Tests** |
| **1. Evoked potentials (VEP ve SEP)** |
| **2. Optic coherence tomography** |
| **3. Urodynamic testing** |
| **4. Cognitive testing** |
| **Other tests for differential diagnosis** |
| **1.Further biochemical tests** (if there is suspicion of vasculitis, wider autoantibody panel, 24-hour urine analysis, GFR evaluation, for rheumatological disorders anti CCP, serum complement levels, for lymphoma serum anti beta2 microglobulins, for sarcoidosis blood and CSF ACE levels, for adrenoleukodystrophy adrenal hormone levels, long/very long chain fatty acids, for mitochondrial diseases serum piruvate, lactate levels, for neuromyelitis optica anti-aquaporin 4 and anti-MOG tests) |
| **2.Specific tests for infectious etiologies** (antibodies for Lyme disease and Brucellosis, PPD and quantiferon tests for tuberculosis) |
| **3.Angiography (cerebral, fluorescein, MRA)** |
| **4.Biopsy** (skin, lymph node, brain and/or leptomeninx, peripheral nerve, other) |
| **5.Eye examination** (retina evaluation for metabolic disorders, uvea evaluation for sarcoidosis and Behçet's disease) |
| **6.Hearing tests** (for Susac) |
| **7.Electrophysiology (nerve conduction studies, EMG)** |
| **8.Chest X-ray** (for chronic latent/sequel infectious lung disorders, and hilar adenopathy) |
| **9.Cardiac examination** (echocardiography for SLE, and mitochondriopathies) |
| **10.Others** (Schirmer test for Sjögren's disease, and salivary gland syntigraphy, for malignancies and metabolic disorders SPECT and PET) |

**Diagnostic Criteria**

Various diagnostic criteria have been developed from the 1950s to the present to increase the sensitivity and specificity of MS diagnosis.[13] The widespread use of MRI as a diagnostic tool, the identification of clinical and imaging prognostic factors, and the increased need for early diagnosis and treatment of MS have led to various revisions in the diagnostic criteria for MS.[121]

In 2001, the International MS Diagnosis Panel, chaired by Ian McDonald, developed new MS diagnosis criteria, now known as the "McDonald criteria".[122] These diagnostic criteria have enabled MRI to be used as a central diagnostic tool in MS diagnosis.[121,122] The 2001 McDonald criteria were last revised in 2017 (Table 2).[123] Every revision of diagnosis criteria over time has enabled MS to be diagnosed earlier and more accurately.[124]

**Table 2.** Revised 2017 McDonald Criteria for MS Diagnosis.[123]

| Number of clinical attacks | Number of lesions with objective clinical findings | Additional data needed for MS diagnosis |
|---|---|---|
| ≥2 attack | ≥2 lesion | – |
| ≥2 attack | 1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location) | – |
| ≥2 attack | 1 lesion | Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI |
| 1 attack | ≥2 lesion | Dissemination in time demonstrated by an additional clinical attack or by MRI or demonstration of CSF-specific oligoclonal bands |
| 1 attack | 1 lesion | Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI and Dissemination in time demonstrated by an additional clinical attack or by MRI or demonstration of CSF-specific oligoclonal bands |

**SYMPTOMS**

MS symptoms vary depending on the location and size of the lesions occurring in CNS.[125] Common symptoms include spasticity, fatigue, sexual and bladder dysfunction, pain, and cognitive impairment (Table 3).[126] Cognitive impairments are especially common in advanced cases, and include memory loss, attention impairment, problem-solving abilities, slowdown in information processing, and difficulties in switching between cognitive tasks.[127]

**Table 3.** Symptoms monitored by retention regions in MS.[17]

| Eclipse Zone | Symptoms |
|---|---|
| Cerebrum | Cognitive impairment<br>Hemisensory and motor<br>Aff ective (mainly depression)<br>Epilepsy (rare)<br>Focal cortical defi cits (rare) |
| Optic nerve | Unilateral painful loss of vision |
| Cerebellum and cerebellar pathways | Tremor<br>Clumsiness and poor balance |
| Brainstem | Diplopia<br>Vertigo<br>Impaired swallowing |
| Spinal cord | Bladder dysfunction<br>Erectile impotence<br>Constipation |
| Other | Fatigue<br>Pain<br>Temperature sensitivity |

**PATHOGENESIS AND PATHOPHYSIOLOGY**

The characteristic pathological feature of MS is the development of focal inflammation and themialistic lesions in the white ventricular regions of the brain, optic nerve and spinal cord. These lesions occur not only in the white vein regions, but also in the intracortical and deep gray vein areas.[128–130] Histologically, there are several basic processes that lead to the formation of these lesions: inflammation, demyelination, astrogliosis, oligodendrosite damage, neurodegeneration and axonal loss and remyelinization.[131] The generally accepted view of the immunopathogenesis of MS includes myelin-specific auto-reactive T cells activated in the peripheral immune system through interaction between environmental triggers and genetic sensitivity.[132] But more and more evidence in recent years has shown that B cells and the humoral immune cells through them and the congenital immune cell (microglia, dendritic cells, macrophages, etc.) also play an important role in the pathogenesis of MS.[133]

**CLINICAL TYPES**

Better understanding of MS and its pathology resulted in the 1996 MS phenotypes not reflecting the recently identified clinical aspects of the disease, which was regulated by the Committee on Clinical Research in 2013 and has been grouped into three main groups: Clinically Isolated Syndrome (CIS), Relapsing-Remitting MS (RRMS), and Progressive MS (PMS) (Secondary- SPMS and Primary-PRMS). At the same time, these main groups are also sub-categories, depending on the activity

and progression of the disease. The term "activity" is used in all forms of the disease and is defined as the onset of a clinical nuisance for a certain period of time, preferably at least one year, or the presence of new T2 or gadolinium-enhancing lesions. The term "progressive" is mainly used for the progressive forms of the disease and refers to the continuous increase in neurological dysfunction/invalidity, fluctuations and stages of stability, which are objectively documented without clinical evaluation at least once a year with no definitive recovery. PMS (whether SPMS or PRMS) has four possible sub-classifications, taking into account the level of disability.[134]

**Clinical Isolated Syndrome (CIS)**

CIS is defined as the first clinical presentation of a disease that shows the characteristics of inflammatory demyelination, which may be MS, but which does not yet meet the criteria for spread over time.[135] Depending on clinical and diagnostic findings, isolated symptoms have a (high/low) risk of transition to MS over time.[136]

**Relapsing- Remitting MS (RRMS)**

RRMS is the most common phenotype of MS, characterized by clearly defined attacks (relapse or exacerbation) and subsequent periods of partial or complete recovery (remission), where new symptoms appear or existing symptoms increase, accounting for approximately 85% of cases. During the period of remission of RRMS, all symptoms may disappear or some symptoms can persist and become permanent, while there is no noticeable progression in the disease.[137]

**Secondary-Progressive MS (SPMS)**

SPMS is a phenotype that follows RRMS, characterized by a decrease in seizures, with no apparent signs of remission, and a gradual worsening of symptoms over time (accumulation of weakness).[138] Studies before approved disease-changing treatments were available have shown that 50% of patients diagnosed with RRMS will transition to SPMS within 10 years and 90% within 25 years.[137,139]

**Primary-Progressive MS (PPMS)**

PPMS is the subtype of MS with the worst prognosis.[140] MS is a phenotype of about 10–15% of the population, characterized by worsening neurological symptoms and accumulation of disability, without attack and remission in the early stages of the disease. Walking problems are common because spinal lesions are more common than brain lesions. PPMS is usually diagnosed between the ages of 40-50 and the number of females/men is approximately equal.[137]

**Radiologically Isolated Syndrome (RIS)**

The term RIS, first defined in 2009,[141] describes patients with accidental MRI abnormalities who have thought of demyelination in the absence of clinical signs or symptoms.[142] Since there is no clinical evidence of the disease and the MRI findings alone are insufficient to diagnose MS, RIS is not considered a separate MS phenotype. However, RIS can raise suspicion of MS depending on the morphology and location of the MRI lesions.[141] Therefore, a RIS patient with no pronounced clinical signs or symptoms that suggest MS should be prospectively monitored.[134]

**TREATMENT**

MS is a disease that has no definitive cure. Although there is no definitive treatment for the disease, significant therapeutic advances have been achieved with molecular immunotherapy approaches such as peptide vaccination, administration of monoclonal antibodies, and immunogenic copolymers. The primary objectives of these therapeutic strategies are to shift the autoimmune response associated with MS to a non-inflammatory T-helper 2 (Th2) cell response, inactivate or heal cytotoxic auto-reactive T cells, induce the secretion of anti-inflammatory cytokines, and prevent the absorption of auto-active lymphocytes into CNS.[143] The goals of MS treatments are to treat specific symptoms, reduce the frequency of recurrences, and prevent the disease from progressing.[144] To achieve these goals, an individualised, dynamic, long-term treatment strategy with continuous monitoring of disease activity should be implemented. The treatment plan should be able to adapt to the changing needs of each patient based on the severity of symptoms, clinical evidence of disease progression, increased disease burden on MRI and the development of neutralizing antibodies (NAb).[145]

Although there are various treatment options available for RRMS, there are still limited treatment options for progressive MS forms (SPMS and PPMS).[146,147] Current treatments are focused on reducing biological activity through treating acute seizures, improving symptoms, and disease-modifying treatments.[148] Emerging therapies include CNS-penetrant Bruton's tyrosine kinase inhibitors and autologous haematopoietic stem cell transplantation, as well as therapies for remyelination or neuroprotection.[149]

**Acute Attack Treatment**

MS attacks are typically defined as a new or worsening neurological deficit that lasts for 24 hours or more in the absence of fever or infection. It is a distinctive feature of MS and is often associated with significant functional impairment and low quality of life.[150] In acute seizures, the most common symptom complexes generally relate to new or worsening inflammatory processes, including optic nerve, spinal cord, cerebellum and/or cerebrum. Therefore, the symptoms may vary, or may be a combination of visual impairment, sensory and motor impairments, balance problems, and cognitive deficiencies.[151,152] Although most MS attacks usually end with a recovery period that leads to clinical remission and sometimes results in complete recovery (especially in the early stages of the disease), symptoms that remain after the attacks can persist and contribute to the gradual progression of the disability.[150] Therefore, seizure treatment is important because it can help shorten and reduce the disability associated with the course of the disease.[153]

Different treatment methods, such as intravenous (IV) corticosteroids, plasma exchange, or adrenocorticotropic hormone (ACTH), are commonly used during periods of MS attacks.[154,155] Although these treatments are effective in reducing the duration of attacks and helping patients to recover faster, they do not have long-term neuro-protective benefits.[156–160] Synthetic forms of corticosteroids, IV and rarely oral, are the

most commonly used drugs in the treatment of seizures. [161–163] Synthetic corticosteroids used in therapy include prednisone, prednisolone, dexamethasone and methylprednisolon.[164] These agents work by preventing edema, stabilizing the blood-brain barrier, reducing pro-inflammatory cytokines, and apoptosis of T cells.[165] Plasmaferesis is a good choice for selected patients who do not respond to corticosteroid therapy and are expected to have permanent absence.[18]

**Symptomatic Treatment**

MS has a wide range of significant and disruptive symptoms, including fatigue, bladder dysfunction, cognitive impairment, pain and spasticity. Symptomatic treatment is aimed at eliminating or reducing symptoms that impair the functional abilities and quality of life of affected patients.[166] An untreated symptom can become worse or lead to other symptoms, causing an interlinked cycle of symptoms.[145]

Immunomodulation or immunosuppression, as well as the specific treatment of symptoms, is an important component of the overall treatment of MS. The methods available for symptomatic treatment are pharmaceutical and non-pharmaceutical. Spasticity, tonic spasms, fatigue, paresthesia, depression, sexual and bladder disorders, are some of the symptoms that require pharmacological intervention.[166]

Spasticity is the main cause of physical disability in MS patients. Non-pharmacological treatment options for spasticity include physician-recommended exercise regimens (stretching exercises to improve flexibility, aerobic exercises, and active and passive movements covering the full range of motion) and relaxation techniques (meditation, yoga).[145] When conditions such as hardness or spasm adversely affect sleep, medication is needed.[167] In pharmacological therapy, baclofen (a GABA receptor stimulant), benzodiazepines and tizanidine (a-adrenergic receptor agonist) are used.[145] The side effects of benzodiazepines, such as sedation and addiction, limit their use in MS.[168,169]

Fatigue is characterized by a lack of energy or a feeling of fatigue in 80% to 97% of MS patients.[170,171] Non-pharmacological treatment of the disease involves treating symptoms that lead to fatigue, such as sleep disorders and depression, and improving the patient's mobility through exercise.[145] In the pharmacological treatment of fatigue, drugs such as modafinil [172] and amantadine [173] are used.

Depression is a symptom that occurs in approximately 50% of MS patients.[174] Treatment of depression is aimed at preventing suicide, as well as reducing depressive emotions through appropriate psychotherapy and, if necessary, medication.[166] Selective serotonin reuptake inhibitors (fluoxetine, sertraline, escitalopram, citalopram and paroxetine), tricyclic antidepressants (nortriptyline and amitriptyline) and atypical antidepressants (venlafaxine and bupropion) are used in the pharmacological treatment of depression.[145]

**Disease Modifying Treatments (DMT)**

Progress in the treatment of MS has been made over the past three decades with the development of novel, highly effective disease-modifying therapies (DMTs) targeting a variety of mechanisms, including immunomodulation, immunosuppression and enhanced immune cell sequestration. (Table 4, 5, 6, 7).[149] DMTs have been significant in the treatment of MS by reducing clinical attacks, slowing the incapacity and progression of the disease, and minimizing the activity of MRI lesions. Currently 24 different DMTs are approved, including injectable, oral and infused drugs.[175] These drugs have different mechanisms of action, methods of administration and frequency, efficacy and safety, which have been shown to be effective in reducing inflammatory activity and relapse rates. DMTs approved for the treatment of RRMS include glatiramer acetate, monoclonal antibodies (alemtuzumab, natalizumab, okrelizumab), mitoxantron, dimethylfumarate, teriflunomide, fingolimod, and cladribine.[176]

**Table 4.** Low, Medium and High Effectiveness Treatments for MS.[177]

| Low-efficacy treatments | Moderate-efficacy treatments | High-efficacy treatments |
|---|---|---|
| • Interferons | • Cladribine* | • Ocrelizumab |
| • Glatiramer acetate | • S1P modulators* | • Ofatumumab |
| • Teriflunomide | • Fumarates | • Natalizumab |
| | | • Alemtuzumab |

* May be considered to have moderate-to-high efficacy.

Interferon beta-1 (IFNβ-1) was the first DMT to be used in the treatment of RRMS.[178,179] The therapeutic mechanism of action in MS is to down-regulate the expression of MHC (major histocompatibility complex) molecules on antigen-presenting cells, suppress pro-inflammatory cytokines, increase anti-inflammatory cytokines, inhibit T cell proliferation and provide immunomodulation by blocking the migration of inflammatory cells to the CNS.[179] Current IFN-β therapies include both subcutaneous and intramuscular formulations with different injection frequencies.[180,181] The most common side effects in IFN-β treatment include injection site reactions and influenza-like symptoms that respond to acetaminophen, ibuprofen and glucocorticoids and tend to decrease over time.[182] Other side effects include exacerbating pre-existing spasticitis, depression, thrombocytopenia, mild anemia, and high transaminase levels. These side effects are usually not severe and rarely lead to discontinuation of treatment.[183]

Glatiramer acetate is a mixture of four amino acids (glutamic acid, alanine, lysine, and tyrosine) that are randomly combined to form an antigenically similar polymer to the basic protein of myelin.[184] Although initially used to induce experimental allergic encephalomyelitis (EAE), purified myelin has been found to prevent EAE after injection of the basic protein.[185] Glatiramer acetate, which has a variety of immunomodulatory effects,

enables this effect by strongly randomly binding to MHC molecules and thereby competing with various myelin antigens for their presentation to T cells, with a strong induction of specific Th2 suppressant cells migrating to the brain, along with the expression of anti-inflammatory cytokines such as IL-10 and TGF-B.[184] It is used in the treatment of recurring forms of MS, including CIS, RRMS, and active SPMS.[182] A phase III dose comparison study showed that both 20 mg and 40 mg doses of glatiramer acetate reduced the annual rate of attacks and the average number of Gd-enhancing lesions.[186] Glatiramer acetate, a generally well-tolerated drug, is not associated with flu-like symptoms.[187] Common adverse reactions include injection area reactions, redness, chest clogging, convulsion, shortness of breath, post-injection anxiety and, less commonly, lipoatrophy, which may rarely be distorting and require discontinuation of treatment.[187,188] Routine laboratory monitoring is not required in patients treated with glatiramer acetate and the development of binding antibodies does not impede the therapeutic effectiveness of the drug.[189]

Fumaric acid esters (FAE) is an orally available drug for the treatment of MS that has been tested in phase II/III MS trials and has proven beneficial effects on relapse rates and MR markers.[190] FAE induces the expression of endogenous antioxidative factors in brain cells by activating the transcription factor nuclear factor E2-related factor 2 (Nrf2).[191] Thus, it can protect CNS from the harmful effects of reactive oxygen intermediates released as part of the inflammatory process of the disease.[192] At the same time, the FAE also inhibits the transcription factor nuclear factor κB (NFκB) by preventing the expression of various inflammatory cytokines, chemokines and adhesion molecules.[193] Dimethyl fumarate, a methyl ester of fumaric acid, is an immunomodulator compound approved for the treatment of RRMS.[182] In two phase III trials of dimethyl fumarate for the treatment of RRMS, there was a 44-53% reduction in the annual relapse rate, a 22-32% reduction in disability progression and a reduction in MR Gd-retaining lesions of up to approximately 75-94%.[194,195] It is initially administered orally at 120 mg twice daily for 7 days, then at 240 mg two times a day. The most common side effects of dimethyl fumarate are gastrointestinal (GIS) symptoms, including diarrhea, nausea, abdominal pain, and flushing. Other side effects include leucopenia, elevated serum aminotransferase and bilirubin levels and proteinuria [182]. Dimethyl fumarate, which is generally well tolerated, has also been associated with some risk of progressive multifocal leukoencephalopathy (PML) in treatment.[196] Most of these cases are lymphopenic, so it is recommended to monitor lymphocyte values every 6-12 months during treatment.[148] Apart from dimethyl fumarate, other oral FAEs used in the treatment of MS include diroximel fumarate and monomethyl fumarate.[149] Diroximel fumarate is an oral bioequivalent compound to dimethyl fumarate, approved by the FDA for the treatment of WMS, RRMS and active SPMS, with fewer GI side effects compared to dimethyl fumarate.[182,197] Monomethyl fumarate is the main active metabolite of dimethyl fumarate and has been approved by the FDA for the treatment of CIS, RRMS and active SPMS. Monomethyl fumarate, treatment is initiated orally at 95 mg twice daily and increased to 190 mg twice daily after 1 week; tolerability profile and monitoring is similar to dimethyl fumarate with improved GIS tolerability.[198]

Teriflunomide is the active metabolite of leflunomide used in the treatment of rheumatoid arthritis.[199] It was approved by the FDA in 2012 for the treatment of recurrent forms of MS.[200] It acts by selectively inhibiting dihydroorotate dehydrogenase, an enzyme important in the synthesis of pyrimidine, and by reducing the proliferation of activated T and B lymphocytes.[199] The TEMSO study showed a relative risk reduction of 31% in the annual relapse rate of the disease, as well as a reduction in disability progression and disease activity.[201] Common side effects of teriflunomide include headache, nausea, diarrhoea, increased hepatic alanine aminotransferase (ALT) and alopecia.[148] Also, teriflunomide carries a black box warning for severe liver damage. Therefore, liver function tests should be followed every month for the first 6 months and then every 6 months. Teriflunomide should not be used in pregnancy or in male or female pregnant patients because it may affect fetal development and may remain in circulation for up to two years after discontinuation of the drug. If necessary, accelerated elimination can be achieved with activated charcoal or cholestyramine.[202]

Cladribine, a synthetic purine nucleoside analogue, is a prodrug that undergoes intracellular phosphorylation by deoxycytidine kinase. The active metabolite (cladribine triphosphate) accumulates in the cell, causing cellular metabolism disruption, DNA damage, and subsequent apoptosis.[203,204] The accumulation of cladribine triphosphate depends on the ratio of 5'-nucleotidases of deoxycitidine kinase.[205] Cladribine preferentially targets lymphocytes due to its relatively high deoxycytidine kinase/5'-nucleotidase ratio and produces rapid and sustained reductions in $CD4^+$ and $CD8^+$ T lymphocytes, with rapid, albeit more transient, effects on $CD19^+$ B lymphocytes.[203,204] Although it causes a decrease in circulating T and B lymphocytes, it also has relatively small and temporary effects on innate immune cells, such as neutrophils and monocytes.[203] An oral medicine approved by the FDA in 2019 for the treatment of relapsing forms of MS excluding CIS.[203,206] Unlike other oral DMTs, it is not taken daily but is dosed by weight (3.5 mg/kg).[206] The oral formulation of cladribine has been developed to be given in short courses over a two-year cycle.[207]

Sphingosine 1-phosphate (S1P) receptor modulators are medicines with a unique mechanism of action for recurrent forms of MS.[208] S1P is a phospholipid with five subtypes found in lymphoid tissue, endothelial cells, flat muscles, atrial myocytes, eyes, and veins. In lymph nodes, the binding of S1P to S1p receptors is important for the output of lymphocytes from the nodes. Binding of S1P receptor modulatory drugs to S1P receptors on lymphocytes leads to altered immune migration, down-regulation of S1P receptor expression and inhibition of lymphocyte egress from lymph nodes. The S1P receptor modulator, fingolimod, was the first oral treatment approved by the FDA in 2010 for the treatment of recurrent forms of MS.[209,210]

Prevents the exit of lymphocytes from secondary lymphoid organs by binding to 4 of the 5 subtypes of S1P receptors (with a higher affinity for S1P receptor type 1).[182] According to clinical studies, fingolimod was superior to both placebo and intramuscular IFNβ-1a in terms of clinical relapse and MRI activity measurements.[211,212] Although it is a generally well-tolerated drug, mild side effects such as elevation or lymphopenia can be observed in liver function tests.[211–214] Other side effects include macular edema, rarely common VZV and cryptococcal infections, PML.[148] Due to the possibility of bradycardia and heart block at the start of fingolimod treatment, a 6-hour observation period (including electrocardiogram monitoring) is recommended for all patients receiving their first dose.[215] In addition, when fingolimod therapy is abruptly discontinued without an effective switch to a new drug, rebound relapses with multiple contrasting lesions or tumefactive lesions may occur after 4-16 weeks.[216,217] Given the favourable profile of fingolimod in the treatment of MS, it has led to the development of small molecule S1P receptor modulators with shorter half-lives, greater S1P receptor selectivity and reduced side effects.[218] Siponimod is an S1P receptor modulator that selectively binds to the S1P receptor-1 and S1P receptor-5 subtypes, inhibiting lymphocyte egress with theoretically fewer off-target effects compared to fingolimod.[219] It was approved by the FDA in 2019 for the treatment of relapsing MS, including active SPMS. It is also the first oral compound approved for active SPMS treatment.[220] Side effects of siponimod include nasopharyngitis, headache, urinary tract infection and falls.[221,222]

Natalizumab, a humanised monoclonal antibody, is the first monoclonal antibody approved in 2004 for the treatment of MS.[223] It acts by inhibiting α4/β1 integrin, a component of VLA-4 (Very Late Activation Antigen-4) expressed by lymphocytes, and preventing its interaction with vascular cell adhesion molecule (VCAM) on endothelial cells. This prevents the lymphocytes from crossing the blood-brain barrier.[182] Natalizumab is highly effective in reducing relapses and slowing disease progression in relapsing forms of MS compared with placebo or IFNβ-1a.[224,225] Despite its well-known effect in reducing disease activity, there is a high risk of developing PML, an infectious and potentially fatal disease of the white matter caused by reactivation of John Cunningham virus (JCV). The risk of developing PML depends on the duration of natalizumab therapy, previous immunosuppressive therapy and exposure to JCV. Therefore, a JCV scan should be performed every 6 months during natalizumab therapy.[226] Another disadvantage of natalizumab is that discontinuation of treatment may trigger 'rebound' disease activity. This is a problem that may be encountered in patients who are non-compliant with treatment, who discontinued treatment before pregnancy or who have JCV seroconversion.[148]

Due to evidence of B-lymphocytes retention in MS pathology, anti-CD20 monoclonal antibodies have begun to be used for the treatment of MS.[19] The antigen binding of anti-CD20 antibodies activates the mechanisms that lead to a decrease in the number of B lymphocytes. Usually, these mechanisms are complement dependent cytotoxicity (CDC) or antibody dependent cell cytotoxin (ADCC). Ublituximab and ocrelizumab have a predominant ADCC effect, which contains natural-killing cells, while ofatumab and rituksimab are a dominant CDC.[227,228] Rituksimab is a chemical monoclonal anti-CD20 antibody widely used in clinical trials against RRMS and PPMS, although it has not received approval from any regulatory agency for the treatment of MS.[148]

Ocrelizumab, a humanised monoclonal antibody targeting the CD20 molecule on the surface of mature B lymphocytes[229], is the first compound approved for the treatment of PPMS.[182] Maintains pre-existing humoural immunity and the regenerative capacity of B lymphocytes by selectively depleting CD20-expressing B lymphocytes.[230,231] It was found to be highly effective in reducing the annual relapse rate compared with IFNβ-1a.[232] In addition, ocrelizumab has been to promote cell death through more ADCC activity and less CDC activity compared to rituximab, and has a more positive antigenic profile.[233–235] When administered as an IV (600 mg) every 6 months, the initial dose is usually divided into 2 infusions of 300 mg at 2 weeks intervals. Common side effects include throat pain, infusion reactions such as redness (more common in the first infusions),[236] respiratory tract and viral infections.[237] Also, although a slight increase in breast cancer risk was in clinical trials, no specific tumor risk was observed without a significant epidemiological inconsistency in the post-marketing analysis.[182]

Ofatumumab, approved for the treatment of RRMS,[238] is a fully humanised anti-CD20 monoclonal antibody that can be administered subcutaneously (a charge dose of 20 mg three times a week, followed by a 20 mg every 4 weeks) with an excellent safety profile and high effectiveness compared to okrelizumab. Common adverse events include injection-related reactions (usually a headache, redness within only 24 hours of the first 1-3 injections), nasopharyngitis, urinary tract infections, and upper respiratory infections.[182]

Alemtuzumab is a humanised monoclonal antibody against the CD52 receptor found on monocytes, lymphocytes and other immune and non-immune cells.[41,239,240]. Alemtuzumab, approved by the FDA in 2014 for use in the treatment of RRMS, is used in patients who often have an inadequate response to 2 or more MS drugs due to the widespread and severe side effects.[241] Alemtuzumab is administered as an IV infusion of 12 mg daily for 5 consecutive days, followed 12 mg daily for 3 consecutive days 12 months later. The main side effects include infections (mainly herpes infections), infusion reactions, autoimmune disorders, including thyroid autoimmunity, and immune thrombocytopenia. Full blood count, kidney, liver and thyroid function should be periodically evaluated for at least 5 years before and after treatment.[182]

Mitoxantron was approved by the FDA in 2000 for the treatment of RRMS and SPMS, but is a drug that is rarely used today due to its side effects. By binding to DNA and causing cross-linking and strand breaks, it reduces the proliferation of macrophages, T and B lymphocytes and also down-regulates the inflammatory cascade.[242] The main side effects include nausea, vomiting, hair loss, leukemia, cardiotoxicity, fatigue, infection, leukopenia and thrombocytopenia.[243]

**Table 5.** Injection treatments for MS.[244]

| Name of the drug | Brand name | Administration Route |
|---|---|---|
| IFNβ-la | Avonex® | Intramuscular |
| IFNβ-la | Rebif® | Subcutaneous |
| PEG-IFNβ-la | Plegridy® | Subcutaneous or Intramuscular |
| IFNβ-lb | Betaseron® | Subcutaneous |
| Glatiramer acetate | Copaxone® | Subcutaneous |
| IFNβ-lb | Extavia® | Subcutaneous |
| Glatiramer acetate | Glatopa® | Subcutaneous |
| Ofatumumab | Kesimpta® | Subcutaneous |

**Table 6.** Oral treatments for MS (National Multiple Sclerosis 2023).[244]

| Name of the drug | Brand name |
|---|---|
| Teriflunomide | Aubagio® |
| Monomethyl fumarate | Bafiertam™ |
| Fingolimod | Gilenya® |
| Cladribine | Mavenclad® |
| Siponimod | Mayzent® |
| Ponesimod | Ponvory™ |
| Fingolimod | Tascenso ODT® |
| Dimethyl fumarate | Tecfidera® |
| Droximel fumarate | Vumerity® |
| Ozanimod | Zeposia® |

**Table 7.** IV infusion treatments for MS.[244]

| Name of the drug | Brand name |
|---|---|
| Ublituximab | Briumvi™ |
| Alemtuzumab | Lemtrada® |
| Mitoxantrone | Novantrone® |
| Ocrelizumab | Ocrevus® |
| Natalizumab | Tysabri® |
| Natalizumab | Tyruko® |

## CONCLUSION

MS is a disease that causes lesion formation in different parts of the CNS with the effect of environmental factors and the individual's lifestyle, decreases the quality of life of individuals due to attacks and can cause permanent disabilities. It usually affects young adults, but can be seen in childhood or older age. In terms of geographical conditions, the prevalence and incidence of the disease varies depending on latitudes. People living in countries closer to the equator have been found to be at lower risk, while those living in higher-level countries are at higher risk. This proves the relationship between exposure to sunlight and/or vitamin D levels and MS. When the etiology of MS is analysed, factors such as genetic predisposition, EBV, smoking, obesity and intestinal microbiota have been found to be effective. Since the location of the lesions forms a wide range in CNS, the neurological manifestations and symptoms of MS show a heterogeneous clinical structure. MS is classified into four categories: CIS, RRMS, PPMS, and SPMS.

Since there are no clinical or laboratory findings to definitively diagnose MS, diagnostic tools such as MRI, CSF analysis and evoked potential tests are used in addition to a comprehensive history and physical examination. MRI is the most important diagnostic and prognostic technique for assessing MS (especially in the early stages of the disease).

Since MS is a progressive neurological disease, it is necessary to make an accurate and early diagnosis and start the right treatment at the right time. Otherwise, the progressive degenerative process occurring in the CNS will create permanent disabilities and negatively affect the quality of life of the person. Exercise is recommended to reduce symptoms such as fatigue and muscle weakness that adversely affect patients' daily activities. At the same time, patients should not consume harmful substances such as smoking, eat regular and healthy foods.

Although there is no definitive treatment for MS, the methods applied for the management of the disease can be categorised into three main groups: Acute relapse management, disease modifying treatment and symptomatic treatment. In acute relapse management, different treatment modalities such as IV corticosteroids, plasma exchange or ACTH are generally used; in symptomatic management, drugs such as baclofen, tizanidine, modafinil, fluoxetine, and in disease-modifying management, drugs such as glatiramer acetate, fingolimod, cladribine, alemtuzumab, ocrelizumab, dimethyl fumarate are used. Currently, CNS-penetran Bruton's tyrosine kinase inhibitors and autologous haematopoietic stem cell transplantation therapies are among the treatment options being investigated for the treatment of MS. Apart from these treatment options, research is ongoing for the definitive treatment of MS.

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## REFERENCES

1. Dobson R, Giovannoni G. Multiple sclerosis–a review. *Eur J Neurol*. 2019;26, 1:27–40.

2. Filippi M, Bar-Or A, Piehl F, et al. Multiple sclerosis. *Nat Rev Dis Prim*. 2018;43.
3. Charcot JM. Leçons de, 1868. Manuscrits des leçons de JM Charcot. Fonds numérisé Charcot. *Bibliothèque de l'Université Pierre & Marie Curie*. Published online 1968.
4. Motl RW, Learmonth YC. Neurological disability and its association with walking impairment in multiple sclerosis: brief review. *Neurodegener Dis Manag*. 2014;4, 6:491–500.
5. Browne P, Chandraratna D, Angood C, et al. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology*. 2014;83(11):1022-1024. doi:10.1212/WNL.0000000000000768
6. M.S. International Federation. Atlas of MS. *Access Date*. 2023;22.
7. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and Treatment of Multiple Sclerosis: A Review. *JAMA*. 2021;325, 8:765–79.
8. Simpson S, Wang W, Otahal P, Blizzard L, Mei IA, Taylor B V. Latitude continues to be significantly associated with the prevalence of multiple sclerosis: an updated meta-analysis. *J Neurol Neurosurg Psychiatry*. 2019;90, 11:1193–200.
9. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. *Ann Neurol Off J Am Neurol Assoc Child Neurol Soc*. 2007;61, 6:504–13.
10. Gandhi R, Laroni A, Weiner HL. Role of the innate immune system in the pathogenesis of multiple sclerosis. *J Neuroimmunol*. 2010;221:1–2, 7–14.
11. Dedoni S, Scherma M, Camoglio C, et al. An overall view of the most common experimental models for multiple sclerosis. *Neurobiol Dis*. 2023;106230.
12. Brownlee WJ, Hardy TA, Fazekas F, Miller DH. Diagnosis of multiple sclerosis: progress and challenges. *Lancet*. 2017;389, 10076:1336–46.
13. Ömerhoca S, Akkaş SY, İçen NK. Multiple sclerosis: diagnosis and differential diagnosis. *Arch Neuropsychiatry*. 2018;55, Suppl:1.
14. Altunrende B, Birday E, Kasap M, Akman Demir G. Fingolimod for the treatment of relapsing-remitting multiple sclerosis. *Turkish J Neurol*. 2017;176-85.
15. Murray TJ. The history of multiple sclerosis: the changing frame of the disease over the centuries. *J Neurol Sci*. 2009;277:3– 8.
16. Eraksoy M, Bulut S, Alp R. *Multipl Sklerosis*. 1th ed. (Ed NTK, M E, eds.). Güneş Tıp Kitabevleri; 2013.
17. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2008;372:1502–17.
18. Sevim S. Multipl skleroz atakları üzerine güncelleme: Tanım, patofizyoloji, özellikler, taklitçiler ve tedavi. *Turk J Neurol*. 2016;22:99–108.
19. Chmielewska N, Szyndler J. Targeting CD20 in multiple sclerosis—review of current treatment strategies. *Neurol Neurochir Pol*. 2023;57:235–42.
20. Leray E, Moreau T, Fromont A, Edan G. Epidemiology of multiple sclerosis. *Rev Neurol (Paris)*. 2016;172(1):3-13. doi:10.1016/j.neurol.2015.10.006
21. Walton C, King R, Rechtman L, et al. Rising prevalence of multiple sclerosis. Published online 2020:1816-1821.
22. Nicoletti A, Patti F, Lo Fermo S, et al. Possible increasing risk of multiple sclerosis in Catania, Sicily. *Neurology*. 2005;65, 8:1259–63.
23. Sellner J, Kraus J, Awad A, Milo R, Hemmer B, Stüve O. The increasing incidence and prevalence of female multiple sclerosis—a critical analysis of potential environmental factors. *Autoimmun Rev*. 2011;10, 8:495–502.
24. Stenager E. A global perspective on the burden of multiple sclerosis. *Lancet Neurol*. 2019;18, 3:227–8.
25. Orton S-M, Herrera BM, Yee IM, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. *Lancet Neurol*. 2006;5, 11:932–6.
26. Padilha IG, Fonseca AP, Pettengill AL, et al. Pediatric multiple sclerosis: from clinical basis to imaging spectrum and differential diagnosis. *Pediatr Radiol*. 2020;50:776–92.
27. Pilotto S, Gencarelli J, Bova S, et al. Etiological research in pediatric multiple sclerosis: A tool to assess environmental exposures. *Pediatr Ital Genet Environ Expo Quest Mult Scler Journal–Experimental, Transl Clin*. 2021;7, 4:20552173211059050.
28. Yan K, Balijepalli C, Desai K, Gullapalli L, Druyts E. Epidemiology of pediatric multiple sclerosis: a systematic literature review and meta-analysis. *Mult Scler Relat Disord*. 2020;44:102260.
29. Immovilli P, Mitri P, Bazzurri V, et al. The Impact of Highly Effective Treatment in Pediatric-Onset Multiple Sclerosis: A Case Series. *Children*. 2022;9:11.
30. Jeong A, Oleske DM, Holman J. Epidemiology of pediatric-onset multiple sclerosis: a systematic review of the literature. *J Child Neurol*. 2019;34, 12:705–12.
31. Hemmer B, Cepok S, Nessler S, Sommer N. Pathogenesis of multiple sclerosis: an update on immunology. *Curr Opin Neurol*. 2002;15, 3:227–31.
32. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Medical progress. *Mult Scler N Engl J Med*. 2000;343:938–52.
33. Smolders J, Damoiseaux J, Menheere P, Hupperts R. Vitamin D as an immune modulator in multiple sclerosis, a review. *J Neuroimmunol*. 2008;194:1–2, 7–17.
34. Potemkowski A. Stwardnienie rozsiane w świecie iw Polsce–ocena epidemiologiczna. *Aktual Neurol*. 2009;2, 9:91–7.
35. Ramagopalan S, Valdar W, Criscuoli M, et al. Age of puberty and the risk of multiple sclerosis: a population based study. *Eur J Neurol*. 2010;16, 3:342–7.
36. Briggs FB, Gunzler DD, Ontaneda D, Marrie RA. Smokers with MS have greater decrements in quality of life and disability than non-smokers. *Mult Scler J*. 2014;23, 13:1772–81.
37. Khan Z, Gupta GD, Mehan S. Cellular and molecular evidence of multiple sclerosis diagnosis and treatment challenges. *J Clin Med*. 2023;12, 13:4274.
38. Van der Mei, I., Lucas RM, Taylor B, et al. Population attributable fractions and joint effects of key risk factors for multiple sclerosis. *Mult Scler J*. 2016;22, 4:461–9.

**39.** Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol*. 2017;13, 1:25–36.

**40.** Jafari N. *Risk Factors in Cause and Course of Multiple Sclerosis*. Doktora Tezi, Erasmus Üniversitesi; 2011.

**41.** Cohen JA, Rae-Grant A. *Handbook of Multiple Sclerosis*. 2th ed. Springer Healthcare Ltd.; 2012. doi:10.1007/978-1-907673-50-4

**42.** Brynedal B, Duvefelt K, Jonasdottir G, et al. HLA-A confers an HLA-DRB1 independent influence on the risk of multiple sclerosis. *PLoS One*. 2007;2, 7:664.

**43.** Sawcer S, Hellenthal G, Pirinen M, et al. International Multiple Sclerosis Genetics Consortium Wellcome Trust Case Control Consortium 2 Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011;476, 7359:214–9.

**44.** Beecham A, Patsopoulos N, Xifara D, et al. International Multiple Sclerosis Genetics Consortium (IMSGC). Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet*. 2013;45, 11:1353–60.

**45.** Moutsianas L, Jostins L, Beecham AH, et al. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat Genet*. 2015;47:1107–1113.

**46.** Nouri H, Tabesh H, Saboori M, et al. Protective and risk factors in multiple sclerosis. *Int J Med Rev*. 2019;6, 2:51–8.

**47.** Zawada M. Potential pathogens in multiple sclerosis (MS. *Adv Hyg Exp Med*. 2012;66:758–70.

**48.** Huang J. Biomarkers and Viral Risk Factors in Multiple Sclerosis. Published online 2022. https://www.proquest.com/docview/2700374980?pq-origsite=gscholar&fromopenview=true&sourcetype=Dissertations & Theses

**49.** Gombash SE, Lee PW, Sawdai E, Lovett-Racke AE. Vitamin D as a risk factor for multiple sclerosis: immunoregulatory or neuroprotective? *Front Neurol*. 2022;13:796933.

**50.** Ascherio A, Munger KL, White R, et al. Vitamin D as an early predictor of multiple sclerosis activity and progression. *JAMA Neurol*. 2014;71, 3:306–314.

**51.** Munger KL, Chitnis T, Ascherio A. Body size and risk of MS in two cohorts of US women. *Neurology*. 2009;73, 19:1543–50.

**52.** Cortese M, Riise T, Bjørnevik K, et al. Timing of use of cod liver oil, a vitamin D source, and multiple sclerosis risk: The EnvIMS study. *Mult Scler J*. 2015;21, 14:1856–64.

**53.** Bjørnevik K, Riise T, Casetta I, et al. Sun exposure and multiple sclerosis risk in Norway and Italy: The EnvIMS study. *Mult Scler J*. 2014;20, 8:1042–9.

**54.** Giovannoni G, Ebers G. Multiple sclerosis: the environment and causation. *Curr Opin Neurol*. 2007;20, 3:261–8.

**55.** Hawkes C. Smoking is a risk factor for multiple sclerosis: a metanalysis. *Mult Scler J*. 2007;13, 5:610–5.

**56.** Handel AE, Williamson AJ, Disanto G, Dobson R, Giovannoni G, Ramagopalan S V. Smoking and multiple sclerosis: an updated meta-analysis. *PLoS One*. 2011;6, 1:16149.

**57.** O'Gorman C, Broadley S. Smoking and multiple sclerosis: evidence for latitudinal and temporal variation. *J Neurol*. 2014;261:1677–83.

**58.** Zhang P, Wang R, Li Z, et al. The risk of smoking on multiple sclerosis: a meta-analysis based on 20,626 cases from case-control and cohort studies. *PeerJ*. 2016;4:1797.

**59.** Poorolajal J, Bahrami M, Karami M, Hooshmand E. Effect of smoking on multiple sclerosis: a meta-analysis. *J Public Health (Bangkok)*. 2017;39, 2:312–20.

**60.** Hedström AK, Hillert J, Olsson T, Alfredsson L. Smoking and multiple sclerosis susceptibility. *Eur J Epidemiol*. 2013;28:867–74.

**61.** Degelman ML, Herman KM. Smoking and multiple sclerosis: a systematic review and meta-analysis using the Bradford Hill criteria for causation. *Mult Scler Relat Disord*. 2017;17:207–16.

**62.** Rosiak K. Czynniki ryzyka i wybrane aspekty psychospołeczne w stwardnieniu rozsianym. Badanie kliniczno-kontrolne, Rozprawa doktorska. *Gdańsk*. 2016;2016:3–11.

**63.** Hedström AK, Ryner M, Fink K, et al. Smoking and risk of treatment-induced neutralizing antibodies to interferon β-1a. *Mult Scler J*. 2014;20, 4:445–50.

**64.** Hedström AK, Lima Bomfim I, Barcellos L, et al. Interaction between adolescent obesity and HLA risk genes in the etiology of multiple sclerosis. *Neurology*. 2014;82, 10:865–72.

**65.** Hernán MA, Jick SS, Logroscino G, Olek MJ, Ascherio A, Jick H. Cigarette smoking and the progression of multiple sclerosis. *Brain*. 2005;128, 6:1461–5.

**66.** O'Gorman C, Lucas R, Taylor B. Environmental Risk Factors for Multiple Sclerosis: A Review with a Focus on Molecular Mechanisms. *Int J Mol Sci*. 2012;13, 9:11718–52.

**67.** Baskara I, Kerbrat S, Dagouassat M, et al. Cigarette smoking induces human CCR6+ Th17 lymphocytes senescence and VEGF-A secretion. *Sci Rep*. 2020;10, 1:6488.

**68.** Alrouji M, Manouchehrinia A, Gran B, Constantinescu CS. Effects of cigarette smoke on immunity, neuroinflammation and multiple sclerosis. *J Neuroimmunol*. 2019;329:24–34.

**69.** Smith KJ, Lassmann H. The role of nitric oxide in multiple sclerosis. *Lancet Neurol*. 2002;1, 4:232–41.

**70.** Lauer K. Environmental risk factors in multiple sclerosis. *Expert Rev Neurother*. 2010;10, 3:421–40.

**71.** Shirani A, Tremlett H. The effect of smoking on the symptoms and progression of multiple sclerosis: a review. *J Inflamm Res*. Published online 2010:115–26.

**72.** Ammitzbøll C, Essen MR, Börnsen L, et al. GPR15+ T cells are Th17 like, increased in smokers and associated with multiple sclerosis. *J Autoimmun*. 2019;97:114–21.

**73.** Gao Z, Nissen JC, Ji K, Tsirka SE. The experimental autoimmune encephalomyelitis disease course is modulated by nicotine and other cigarette smoke components. *PLoS One*. 2014;9, 9:107979.

**74.** Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, Brenner T. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via

suppression of Th1 and Th17 responses. *J Immunol*. 2009;183, 10:6681–8.

**75.** Alfredsson L, Olsson T. Lifestyle and environmental factors in multiple sclerosis. *Cold Spring Harb Perspect Med*. 2019;9:4.

**76.** Hedström A, Olsson T, Alfredsson L. Smoking is a major preventable risk factor for multiple sclerosis. *Mult Scler J*. 2016;22(8):1021-1026. doi:10.1177/1352458515609794

**77.** Munger KL. Childhood obesity is a risk factor for multiple sclerosis. *Mult Scler J*. 2013;19:13.

**78.** Wesnes K, Riise T, Casetta I, et al. Body size and the risk of multiple sclerosis in Norway and Italy: the EnvIMS study. *Mult Scler J*. 2015;21, 4:388–95.

**79.** De Rosa, V., Procaccini C, Calì G, et al. A key role of leptin in the control of regulatory T cell proliferation. *Immunity*. 2007;26, 2:241–55.

**80.** Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA. Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology*. 1997;138, 2:855–8.

**81.** Matkovic V, Ilich JZ, Skugor M, et al. Leptin is inversely related to age at menarche in human females. *J Clin Endocrinol Metab*. 1997;82, 10:3239–45.

**82.** Ramagopalan S V, Dobson R, Meier UC, Giovannoni G. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. *Lancet Neurol*. 2009;9, 7:727–39.

**83.** Chitnis T. Role of puberty in multiple sclerosis risk and course. *Clin Immunol*. 2013;149, 2:192–200.

**84.** Lee JM, Appugliese D, Kaciroti N, Corwyn RF, Bradley RH, Lumeng JC. Weight status in young girls and the onset of puberty. *Pediatrics*. 2007;119, 3:624– 30.

**85.** Gianfrancesco MA, Barcellos LF. Obesity and multiple sclerosis susceptibility: a review. *J Neurol neuromedicine*. 2016;1, 7:1.

**86.** Abna Z, Fazeli SA, Mirhashemi S, et al. A narrative review study on the effects of obesity and bariatric surgery on multiple sclerosis. *Ann Indian Acad Neurol*. 2021;24, 5:664.

**87.** Matarese G, Carrieri PB, Cava A, et al. Leptin increase in multiple sclerosis associates with reduced number of CD4+ CD25+ regulatory T cells. *Proc Natl Acad Sci*. 2005;102, 14:5150–5.

**88.** Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest*. 2007;117, 1:175–84.

**89.** Procaccini C, Pucino V, Mantzoros CS, Matarese G. Leptin in autoimmune diseases. *Metabolism*. 2015;64, 1:92–104.

**90.** Matarese G, Carrieri PB, Montella S, Rosa V, Cava A. Leptin as a metabolic link to multiple sclerosis. *Nat Rev Neurol*. 2010;6, 8:455–61.

**91.** Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr*. 2000;72(3):690–693.

**92.** Mokry LE, Ross S, Timpson NJ, Sawcer S, Davey Smith G, Richards JB. Obesity and multiple sclerosis: a mendelian randomization study. *PLoS Med*. 2016;13, 6:1002053.

**93.** Locke AE, Kahali B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518, 7538:197–206.

**94.** Gianfrancesco MA, Stridh P, Rhead B, et al. Evidence for a causal relationship between low vitamin D, high BMI, and pediatric-onset MS. *Neurology*. 2017;88, 17:1623–9.

**95.** Handel AE, Giovannoni G, Ebers GC, Ramagopalan S V. Environmental factors and their timing in adult-onset multiple sclerosis. *Nat Rev Neurol*. 2010;6, 3:156–66.

**96.** DeLuca H, Plum L. UVB radiation, vitamin D and multiple sclerosis. *Photochem Photobiol Sci*. 2017;16:411–5.

**97.** Gale CR, Martyn CN. Migrant studies in multiple sclerosis. *Prog Neurobiol*. 1995;47:4–5, 425–48.

**98.** Sabel CE, Pearson JF, Mason DF, Willoughby E, Abernethy DA, Taylor B V. The latitude gradient for multiple sclerosis prevalence is established in the early life course. *Brain*. 2021;144, 7:2038–46.

**99.** Tao C, Simpson S, Mei I, et al. Higher latitude is significantly associated with an earlier age of disease onset in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2016;87, 12:1343–9.

**100.** Hucke S, Eschborn M, Liebmann M, et al. Sodium chloride promotes pro-inflammatory macrophage polarization thereby aggravating CNS autoimmunity. *J Autoimmun*. 2016;67:90–101.

**101.** Farez MF, Fiol MP, Gaitán MI, Quintana FJ, Correale J. Sodium intake is associated with increased disease activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2015;86, 1:26–31.

**102.** Wu C, Yosef N, Thalhamer T, et al. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature*. 2013;496, 7446:513–7.

**103.** Kleinewietfeld M, Manzel A, Titze J, et al. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature*. 2013;496, 7446:518–22.

**104.** Chen J, Chia N, Kalari KR, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep*. 2016;6, 1:1–10.

**105.** Galluzzo P, Capri FC, Vecchioni L, et al. Comparison of the intestinal microbiome of Italian patients with multiple sclerosis and their household relatives. *Life*. 2021;11, 7:620.

**106.** Lin X, Liu Y, Ma L, et al. Constipation induced gut microbiota dysbiosis exacerbates experimental autoimmune encephalomyelitis in C57BL/6 mice. *J Transl Med*. 2021;19:1–16.

**107.** Haase S, Haghikia A, Wilck N, Müller DN, Linker RA. Impacts of microbiome metabolites on immune regulation and autoimmunity. *Immunology*. 2018;154, 2:230–8.

**108.** Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. *Nat Rev Gastroenterol Hepatol*. 2019;16, 1:35–56.

**109.** Krautkramer KA, Fan J, Bäckhed F. Gut microbial metabolites as multi-kingdom intermediates. *Nat Rev Microbiol*. 2021;19, 2:77–94.

**110.** Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+ CD25+ regulatory T cells in patients with multiple sclerosis. *J Exp Med*. 2004;199,

7:971–9.
**111.** Tomé-Carneiro J, Gonzálvez M, Larrosa M, et al. Resveratrol in primary and secondary prevention of cardiovascular disease: a dietary and clinical perspective. *Ann N Y Acad Sci*. 2013;1:37–51.
**112.** Andersen C, Søndergaard HB, Bang Oturai D, et al. Alcohol consumption in adolescence is associated with a lower risk of multiple sclerosis in a Danish cohort. *Mult Scler J*. 2019;25, 12:1572–9.
**113.** Berer K, Gerdes LA, Cekanaviciute E, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci*. 2017;114, 40:10719–24.
**114.** Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*. 2006;444, 7117:337–42.
**115.** Fonseca-Kelly Z, Nassrallah M, Uribe J, et al. Resveratrol neuroprotection in a chronic mouse model of multiple sclerosis. *Front Neurol*. 2012;3:84.
**116.** Imler Jr TJ PTM. Decreased severity of experimental autoimmune encephalomyelitis during resveratrol administration is associated with increased IL-17+ IL-10+ T cells, CD4− IFN-γ+ cells, and decreased macrophage IL-6 expression. *Int Immunopharmacol*. 2009;9, 1:134–43.
**117.** Hedström A, Mowry E, Gianfrancesco M, et al. High consumption of coffee is associated with decreased multiple sclerosis risk; results from two independent studies. *J Neurol Neurosurg Psychiatry*. 2016;87, 5:454–60.
**118.** Reich DS, Lucchinetti CF, Calabresi PA. Multiple Sclerosis. *N Engl J Med*. 2018;378:169–180.
**119.** Peschl P, Bradl M, Höftberger R, Berger T, Reindl M. Myelin oligodendrocyte glycoprotein: deciphering a target in inflammatory demyelinating diseases. *Front Immunol*. 2017;8:529.
**120.** McCreary M, Mealy M, Wingerchuk D, Levy M, DeSena A, Greenberg B. Updated diagnostic criteria for neuromyelitis optica spectrum disorder: Similar outcomes of previously separate cohorts. *Mult Scler Journal–Experimental, Transl Clin*. 2018;4, 4:2055217318815925.
**121.** Milo R, Miller A. Revised diagnostic criteria of multiple sclerosis. *Autoimmun Rev*. 2014;13:4–5, 518–24.
**122.** McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol Off J Am Neurol Assoc Child Neurol Soc*. 2001;50, 1:121–7.
**123.** Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018;17, 2:162–73.
**124.** Brownlee WJ, Swanton JK, Altmann DR, Ciccarelli O, Miller DH. Earlier and more frequent diagnosis of multiple sclerosis using the McDonald criteria. *J Neurol Neurosurg Psychiatry*. 2015;86, 5:584–5.
**125.** Friedrich A. Multiple Sklerose erkennen. *Heilberufe*. 2021;73, 10:26–9.
**126.** Crayton H, Heyman RA, Rossman HS. A multimodal approach to managing the symptoms of multiple sclerosis. *Neurology*. 2004;63, 11 sup:12– 8.
**127.** Deangelis TM, Miller A. Diagnosis of multiple sclerosis. In: Goodin DS, ed. *Handbook of Clinical Neurology: Multiple Sclerosis and Related Disorders*. 3rd series. The; 2014:317–342.
**128.** Brownell B, Hughes JT. The distribution of plaques in the cerebrum in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 1962;25, 4:315.
**129.** Arrambide G, Rovira A, Sastre-Garriga J, et al. Spinal cord lesions: a modest contributor to diagnosis in clinically isolated syndromes but a relevant prognostic factor. *Mult Scler J*. 2018;24, 3:301–12.
**130.** Riederer I, Mühlau M, Hoshi M-M, Zimmer C, Kleine JF. Detecting optic nerve lesions in clinically isolated syndrome and multiple sclerosis: double-inversion recovery magnetic resonance imaging in comparison with visually evoked potentials. *J Neurol*. 2019;266:148–56.
**131.** Kuhlmann T, Lassmann H, Brück W. Diagnosis of inflammatory demyelination in biopsy specimens: a practical approach. *Acta Neuropathol*. 2008;115:275–87.
**132.** Inglese M. Multiple sclerosis: new insights and trends. *Am J Neuroradiol*. 2006;27, 5:954–7.
**133.** Ma X, Ma R, Zhang M, Qian B, Wang B, Yang W. Recent progress in multiple sclerosis treatment using immune cells as targets. *Pharmaceutics*. 2023;15, 3:728.
**134.** Lublin FD. New multiple sclerosis phenotypic classification. *Eur Neurol*. 2014;72, Suppl.:1–5.
**135.** Miller D, Barkhof F, Montalban X, Thompson A, Filippi M. Clinically isolated syndromes suggestive of multiple sclerosis, part I: natural history, pathogenesis, diagnosis, and prognosis. *Lancet Neurol*. 2005;4, 5:281–8.
**136.** Wiendl H, Gold R, Berger T, et al. No Title. 2021;14:17562864211039648.
**137.** National Multiple Sklerosis Society. Access Date, 27 February 2024. Link, https://www.nationalCNSociety.org/What-is-MS/Types-of-MS/Primary-progressive-MS. Published online 2024.
**138.** MS Australia Research Advocacy Cure. Access Date, 26 February 2024. Link, https://www.msaustralia.org.au/types-of-ms/. Published online 2024.
**139.** National Multiple Sklerosis Society. Link, https://www.nationalCNSociety.org/What-is-MS/Types-of-MS/Secondary-progressive-MS. Published online 2024.
**140.** Koch M, Kingwell E, Rieckmann P, Tremlett H. The natural history of primary progressive multiple sclerosis. *Neurology*. 2009;73, 23:1996–2002.
**141.** Okuda D, Mowry E, Beheshtian A, et al. Incidental MRI anomalies suggestive of multiple sclerosis: the radiologically isolated syndrome. *Neurology*. 2009;72, 9:800–5.
**142.** Klineova S, Lublin FD. Clinical course of multiple sclerosis. *Cold Spring Harb Perspect Med*. 2018;8, 9:28928.
**143.** Metaxakis A, Petratou D, Tavernarakis N. Molecular interventions towards multiple sclerosis treatment. *Brain*

*Sci*. 2020;10:5,299.

**144.** Cudalba D, Gica N, Peltecu G, Botezatu R, Panaitescu AM. Multiple sclerosis in pregnancy. Treatment options and outcomes: a review. *Rom J Neurol*. 2022;21, 2:119.

**145.** Johnson B, Maves T, Mazanec WJ, Miller JR. Stepped-care approach to treating MS: a managed care treatment algorithm. *J Manag Care Pharm*. 2004;10, 3:26– 32.

**146.** Faissner S, Plemel JR G, R Y, V.W. Progressive multiple sclerosis: from pathophysiology to therapeutic strategies. *Nat Rev drug Discov*. 2019;18, 12:905–22.

**147.** Brummer T, Zipp F, Bittner S. T cell–neuron interaction in inflammatory and progressive multiple sclerosis biology. *Curr Opin Neurobiol*. 2022;75:102588.

**148.** Hauser SL, Cree BA. Treatment of multiple sclerosis: a review. *Am J Med*. 2020;133, 12:1380–90.

**149.** Yang JH, Rempe T, Whitmire N, Dunn-Pirio A, Graves JS. Therapeutic advances in multiple sclerosis. *Front Neurol*. 2022;13:824926.

**150.** Lublin FD, Baier M, Cutter G. Effect of relapses on development of residual deficit in multiple sclerosis. *Neurology*. 2003;61, 11:1528–32.

**151.** Frohman EM, Shah A, Eggenberger E, Metz L, Zivadinov R, Stüve O. Corticosteroids for multiple sclerosis: I. *Appl Treat exacerbations Neurother*. 2007;4:618–26.

**152.** Repovic P, Lublin FD. Treatment of multiple sclerosis exacerbations. *Neurol Clin*. 2011;29, 2:389–400.

**153.** Berkovich R. Treatment of acute relapses in multiple sclerosis. In: *Translational Neuroimmunology in Multiple Sclerosis*. ; 2016:307–26.

**154.** Citterio A, Mantia L, Ciucci G, et al. Corticosteroids or ACTH for acute exacerbations in multiple sclerosis. *Cochrane database Syst Rev*. Published online 1996:11.

**155.** Diebold M, Derfuss T. Immunological treatment of multiple sclerosis. In: *Seminars in Hematology*. ; 2016:54– 7.

**156.** Havrdova E, Zivadinov R, Krasensky J, et al. Randomized study of interferon beta-1a, low-dose azathioprine, and low-dose corticosteroids in multiple sclerosis. *Mult Scler J*. 2009;15, 8:965–76.

**157.** Morrow S, Metz L, Kremenchutzky M. High dose oral steroids commonly used to treat relapses in Canadian MS clinics. *Can J Neurol Sci*. 2009;36, 2:213–5.

**158.** Myhr K, Mellgren S. Corticosteroids in the treatment of multiple sclerosis. *Acta Neurol Scand*. 2009;120:73–80.

**159.** Van Der Voort LF, Visser A, Knol DL, Oudejans CBM, Polman CH, Killestein J. Lack of interferon-beta bioactivity is associated with the occurrence of relapses in multiple sclerosis. *Eur J Neurol*. 2009;16(9):1049-1052. doi:10.1111/j.1468-1331.2009.02649.x

**160.** Inglese M, Petracca M. Therapeutic strategies in multiple sclerosis: a focus on neuroprotection and repair and relevance to schizophrenia. *Schizophr Res*. 2015;161, 1:94–101.

**161.** Durelli L, Cocito D, Riccio A, et al. High-dose intravenous methylprednisolone in the treatment of multiple sclerosis: Clinical-immunologic correlations. *Neurology*. 1986;36, 2:238.

**162.** Beck RW, Cleary PA, Anderson JMM, et al. A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis. *N Engl J Med*. 1992;326, 9:581–8.

**163.** Milligan N, Newcombe R, Compston D. A double-blind controlled trial of high dose methylprednisolone in patients with multiple sclerosis: 1. Clinical effects. *J Neurol Neurosurg Psychiatry*. 1987;50, 5:511–6.

**164.** Goodin DS, Reder AT, Bermel RA, et al. Relapses in multiple sclerosis: Relationship to disability. *Mult Scler Relat Disord*. 2016;6:10–20.

**165.** Gold R, Buttgereit F, Toyka K V. Mechanism of action of glucocorticosteroid hormones: possible implications for therapy of neuroimmunological disorders. *J Neuroimmunol*. 2001;117:1–2, 1–8.

**166.** Henze T, Rieckmann P, Toyka K. Symptomatic Treatment of Multiple Sclerosis: Multiple Sclerosis Therapy Consensus Group (MSTCG) of the German Multiple Sclerosis Society1. *Eur Neurol*. 2006;56, 2:78–105.

**167.** Cutter NC, Scott DD, Johnson JC, Whiteneck G. Gabapentin effect on spasticity in multiple sclerosis: a placebo-controlled, randomized trial. *Arch Phys Med Rehabil*. 2000;81, 2:164–9.

**168.** Paisley S, Beard S, Hunn A, Wight J. Clinical effectiveness of oral treatments for spasticity in multiple sclerosis: a systematic review. *Mult Scler J*. 2002;8, 4:319–29.

**169.** Shakespeare D, Boggild M, Young CA, Sclerosis CM, RDotC G. Anti-spasticity agents for multiple sclerosis. *Cochrane Database Syst Rev*. 2003;1.

**170.** Krupp LB. Mechanisms, measurement, and management of fatigue in multiple sclerosis. In: Eds TAJ, C P, R H, eds. *Multiple Sclerosis: Clinical Challenges and Controversies*. 1th ed. Martin Dunitz; 1997:283–94.

**171.** Edgley K, Sullivan MJ, Dehoux E. A survey of multiple sclerosis: II. *Determ Employ status Can J Rehabil*. 1991;4(3):127–132.

**172.** Rammohan K, Rosenberg J, Lynn D, Blumenfeld A, Pollak C, Nagaraja H. Efficacy and safety of modafinil (Provigil®) for the treatment of fatigue in multiple sclerosis: a two centre phase 2 study. *J Neurol Neurosurg Psychiatry*. 2002;72, 2:179–83.

**173.** Krupp LB, Rizvi SA. Symptomatic therapy for underrecognized manifestations of multiple sclerosis. *Neurology*. 2002;58, 8_supp:32– 9.

**174.** Chwastiak L, Ehde DM, Gibbons LE, Sullivan M, Bowen JD, Kraft GH. Depressive symptoms and severity of illness in multiple sclerosis: epidemiologic study of a large community sample. *Am J Psychiatry*. 2002;159, 11:1862–8.

**175.** National Multiple Sklerosis Society. Disease-modifying therapies for MS. Access Date, 25 April 2024. Link, https://nms2cdn.azureedge.net/cCNSite/nationalCNSociety/media/msnationalfiles/brochures/brochure-the-ms-disease-modifying-medications.pdf. Published online 2023.

**176.** Brancati S, Gozzo L, Longo L, Vitale DC, Drago F. Rituximab in multiple sclerosis: are we ready for regulatory approval? *Front Immunol*. 2021;12:661882.

**177.** Samjoo IA, Worthington E, Drudge C, et al. Efficacy

classification of modern therapies in multiple sclerosis. *J Comp Eff Res*. 2021;10, 6:495–507.

**178.** Kappos L, Polman C, Freedman M, et al. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. *Neurology*. 2006;67, 7:1242–9.

**179.** Kieseier BC. The mechanism of action of interferon-β in relapsing multiple sclerosis. *CNS Drugs*. 2011;25:491–502.

**180.** Dhib-Jalbut S, Marks S. Interferon-β mechanisms of action in multiple sclerosis. *Neurology*. 2010;74, 1_supp:17– 24.

**181.** Jakimovski D, Kolb C, Ramanathan M, Zivadinov R, Weinstock-Guttman B. Interferon β for multiple sclerosis. *Cold Spring Harb Perspect Med*. 2018;8, 11:32003.

**182.** Callegari I, Derfuss T, Galli E. Update on treatment in multiple sclerosis. *Presse Med*. 2021;50, 2:104068.

**183.** Calabresi PA. Diagnosis and management of multiple sclerosis. *Am Fam Physician*. 2004;70, 10:1935–44.

**184.** Arnon R, Aharoni R. Mechanism of action of glatiramer acetate in multiple sclerosis and its potential for the development of new applications. *Proc Natl Acad Sci*. 2004;101, suppl:14593–8.

**185.** Teitelbaum D, Meshorer A, Hirshfeld T, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. *Eur J Immunol*. 1971;1, 4:242–8.

**186.** Comi G, Cohen JA, Arnold DL, Wynn D, Filippi M, Group FS. Phase III dose-comparison study of glatiramer acetate for multiple sclerosis. *Ann Neurol*. 2011;69, 1:75–82.

**187.** Johnson K, Brooks B, Cohen J, et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind, placebo-controlled trial. *Neurology*. 1995;45, 7:1268–76.

**188.** Johnson K, Brooks B, Cohen J, et al. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. *Neurology*. 1998;50, 3:701–8.

**189.** Brenner T, Arnon R, Sela M, et al. Humoral and cellular immune responses to Copolymer 1 in multiple sclerosis patients treated with Copaxone®. *J Neuroimmunol*. 2001;115:1–2, 152–60.

**190.** Lee D-H, Gold R, Linker RA. Mechanisms of oxidative damage in multiple sclerosis and neurodegenerative diseases: therapeutic modulation via fumaric acid esters. *Int J Mol Sci*. 2012;13, 9:11783–803.

**191.** Lukashev M, Zeng W, Goelz S, et al. Activation of Nrf2 and modulation of disease progression in EAE models by BG00012 (dimethyl fumarate) suggests a novel mechanism of action combining anti-inflammatory and neuroprotective modalities. *Mult Scler*. Published online 2007:149–.

**192.** Sorensen PS, Sellebjerg F. Oral fumarate for relapsing-remitting multiple sclerosis. *Lancet*. 2008;372, 9648:1447–8.

**193.** Gerdes S, Shakery K, Mrowietz U. Dimethylfumarate inhibits nuclear binding of nuclear factor κB but not of nuclear factor of activated T cells and CCAAT/enhancer binding protein β in activated human T cells. *Br J Dermatol*. 2007;156, 5:838–42.

**194.** Gold R, Kappos L, Arnold DL, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. *N Engl J Med*. 2012;367, 12:1098–107.

**195.** Havrdova E, Hutchinson M, Kurukulasuriya NC, et al. Oral BG-12 (dimethyl fumarate) for relapsing–remitting multiple sclerosis: a review of DEFINE and CONFIRM: Evaluation of: Gold. RJ F, DH M, JT P, eds. *N Engl J Med*. 2013;367:1098–107.

**196.** Mills EA, Mao-Draayer Y. Aging and lymphocyte changes by immunomodulatory therapies impact PML risk in multiple sclerosis patients. *Mult Scler J*. 2018;24, 8:1014–22.

**197.** Naismith RT, Wundes A, ZieCNSen T, et al. Diroximel fumarate demonstrates an improved gastrointestinal tolerability profile compared with dimethyl fumarate in patients with relapsing–remitting multiple sclerosis: results from the randomized, double-blind, phase III EVOLVE-MS-2 study. *CNS Drugs*. 2020;34:185–96.

**198.** Wynn D, Lategan TW, Sprague TN, Rousseau FS, Fox EJ. Monomethyl fumarate has better gastrointestinal tolerability profile compared with dimethyl fumarate. *Mult Scler Relat Disord*. 2020;45:102335.

**199.** Bar-Or A, Pachner A, Menguy-Vacheron F, Kaplan J, Wiendl H. Teriflunomide and its mechanism of action in multiple sclerosis. *Drugs*. 2014;74:659–74.

**200.** US Food and Drug Administration Medication Guide. Published online 2013.

**201.** O'Connor P, Wolinsky JS, Confavreux C, et al. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. *N Engl J Med*. 2011;365, 14:1293–303.

**202.** O'Connor P, Comi G, Freedman MS, et al. Long-term safety and efficacy of teriflunomide: nine-year follow-up of the randomized TEMSO study. *Neurology*. 2016;86, 10:920–30.

**203.** Beutler E. Cladribine (2-chlorodeoxyadenosine. *Lancet*. 1992;340, 8825:952–6.

**204.** Rice GP, Filippi M, Comi G, Group CCS, FtCMS G. Cladribine and progressive MS: clinical and MRI outcomes of a multicenter controlled trial. *Neurology*. 2000;54, 5:1145–55.

**205.** Kawasaki H, Carrera C, Piro L, Saven A, Kipps T, Carson D. Relationship of deoxycytidine kinase and cytoplasmic 5'-nucleotidase to the chemotherapeutic efficacy of 2-chlorodeoxyadenosine. *Blood*. 1993;81(3):597-601. doi:10.1182/blood.V81.3.597.597

**206.** Giovannoni G, Comi G, Cook S, et al. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. *N Engl J Med*. 2010;362, 5:416–26.

**207.** Giovannoni G. Cladribine to treat relapsing forms of multiple sclerosis. *Neurotherapeutics*. 2017;14, 4:874–87.

**208.** Mehling M, Kappos L, Derfuss T. Fingolimod for multiple sclerosis: mechanism of action, clinical outcomes, and future directions. *Curr Neurol Neurosci Rep*. 2011;11:492–7.

**209.** Brinkmann V, Davis MD, Heise CE, et al. The immune modulator FTY720 targets sphingosine 1-phosphate

receptors. *J Biol Chem*. 2002;277, 24:21453–7.
**210.** Mandala S, Hajdu R, Bergstrom J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science*. 2002;296, 5566:346–9.
**211.** Cohen JA, Barkhof F, Comi G, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med*. 2010;362, 5:402–15.
**212.** Kappos L, Radue E-W, O'Connor P, et al. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med*. 2010;362, 5:387–401.
**213.** Calabresi PA, Radue E-W, Goodin D, et al. Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Neurol*. 2014;13, 6:545–56.
**214.** Kappos L, Cohen J, Collins W, et al. Fingolimod in relapsing multiple sclerosis: an integrated analysis of safety findings. *Mult Scler Relat Disord*. 2014;3, 4:494–504.
**215.** Laroni A, Brogi D, Morra V, et al. Safety of the first dose of fingolimod for multiple sclerosis: results of an open-label clinical trial. *BMC Neurol*. 2014;14:65.
**216.** Hatcher SE, Waubant E, Nourbakhsh B, Crabtree-Hartman E, Graves JS. Rebound syndrome in patients with multiple sclerosis after cessation of fingolimod treatment. *JAMA Neurol*. 2016;73, 7:790–4.
**217.** Barry B, Erwin AA, Stevens J, Tornatore C. Fingolimod rebound: a review of the clinical experience and management considerations. *Neurol Ther*. 2019;8:241–50.
**218.** Subei AM, Cohen JA. Sphingosine 1-phosphate receptor modulators in multiple sclerosis. *CNS Drugs*. 2015;29, 7:565–75.
**219.** Gergely P, Nuesslein-Hildesheim B, Guerini D, et al. The selective sphingosine 1-phosphate receptor modulator BAF312 redirects lymphocyte distribution and has species-specific effects on heart rate. *Br J Pharmacol*. 2012;167, 5:1035–47.
**220.** Derfuss T, Mehling M, Papadopoulou A, Bar-Or A, Cohen JA, Kappos L. Advances in oral immunomodulating therapies in relapsing multiple sclerosis. *Lancet Neurol*. 2020;19, 4:336–47.
**221.** Kappos L, Bar-Or A, Cree BA, et al. Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. *Lancet*. 2018;391, 10127:1263–73.
**222.** Cao L, Li M, Yao L, et al. Siponimod for multiple sclerosis. *Cochrane Database Syst Rev*. 2021;11:13647.
**223.** Yaldizli Ö, Putzki N. Natalizumab in the treatment of multiple sclerosis. *Ther Adv Neurol Disord*. 2009;2, 2:115–28.
**224.** Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med*. 2006;354, 9:899–910.
**225.** Rudick RA, Stuart WH, Calabresi PA, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med*. 2006;354, 9:911–23.
**226.** Ryerson LZ, Foley J, Chang I, et al. Risk of natalizumab-associated PML in patients with MS is reduced with extended interval dosing. *Neurology*. 2019;93, 15:1452–62.
**227.** Beum P V, Lindorfer MA, Beurskens F, et al. Complement activation on B lymphocytes opsonized with rituximab or ofatumumab produces substantial changes in membrane structure preceding cell lysis. *J Immunol*. 2008;181, 1:822–32.
**228.** Montalvao F, Garcia Z, Celli S, et al. The mechanism of anti-CD20–mediated B cell depletion revealed by intravital imaging. *J Clin Invest*. 2013;123, 12:5098–103.
**229.** Sorensen PS, Blinkenberg M. The potential role for ocrelizumab in the treatment of multiple sclerosis: current evidence and future prospects. *Ther Adv Neurol Disord*. 2016;9, 1:44–52.
**230.** Sabatino Jr JJ, Pröbstel, A-K., Zamvil SS. B cells in autoimmune and neurodegenerative central nervous system diseases. *Nat Rev Neurosci*. 2019;20, 12:728–45.
**231.** Li R, Patterson KR, Bar-Or A. Reassessing B cell contributions in multiple sclerosis. *Nat Immunol*. 2018;19, 7:696–707.
**232.** Hauser SL, Bar-Or A, Comi G, et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. *N Engl J Med*. 2017;376, 3:221–34.
**233.** Klein C, Lammens A, Schäfer W, et al. Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. MAbs. Published online 2013:22–33.
**234.** Feng JJ, Ontaneda D. Treating primary-progressive multiple sclerosis: potential of ocrelizumab and review of B-cell therapies. *Degener Neurol Neuromuscul Dis*. 2017;7:31–45.
**235.** Gelfand JM, Cree BA, Hauser SL. Ocrelizumab and other CD20+ B-cell-depleting therapies in multiple sclerosis. *Neurotherapeutics*. 2017;14, 4:835–41.
**236.** Hartung H-P. Ocrelizumab shorter infusion: primary results from the ENSEMBLE PLUS substudy in patients with MS. *Neurol Neuroimmunol Neuroinflammation*. 2020;7:807.
**237.** Hauser SL, Kappos L, Montalban X, et al. Safety of ocrelizumab in multiple sclerosis: updated analysis in patients with relapsing and primary progressive multiple sclerosis. *Mult Scler Relat Disord*. 2018;26:264.
**238.** Hauser SL, Bar-Or A, Cohen JA, et al. Ofatumumab versus teriflunomide in multiple sclerosis. *N Engl J Med*. 2020;383, 6:546–57.
**239.** Cohen JA, Coles AJ, Arnold DL, et al. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet*. 2012;380, 9856:1819–28.
**240.** Coles AJ, Twyman CL, Arnold DL, et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet*. 2012;380, 9856:1829–39.
**241.** Dargahi N, Katsara M, Tselios T, et al. Multiple sclerosis: immunopathology and treatment update. *Brain Sci*. 2017;7, 7:78.
**242.** Jeffery DR, Herndon R. Review of mitoxantrone in the

treatment of multiple sclerosis. *Neurology*. 2004;63, 12_sup:19– 24.

**243.** Eckstein C, Bhatti MT. Currently approved and emerging oral therapies in multiple sclerosis: An update for the ophthalmologist. *Surv Ophthalmol*. 2016;61, 3:318–32.

**244.** National Multiple Sklerosis Society. Access Date, 26 February 2024. Link, https://www.nationalCNSociety.org/What-is-MS/Types-of-MS/Relapsing-remitting-MS. Published online 2023.